USER
MANUAL

Version 2.0 for Microsoft® Windows

MasterPlex™ GT 2.0
Genotype Analysis Software

MiraiBio

A HITACHI SOFTWARE COMPANY

For Research Use Only

1201 Harbor Bay Parkway
Suite 150
Alameda, CA 94502

TELEPHONE
1.800.624.6176
1.510.337.2000

FACSIMILE
1.510.337.2099

Part no. P-33120-10250

License Agreement

BEFORE OPENING THIS PACKAGE, YOU SHOULD CAREFULLY READ THE FOLLOWING TERMS AND CONDITIONS. BY OPENING THIS PACKAGE YOU AGREE TO BECOME BOUND BY THE TERMS AND CONDITIONS OF THIS AGREEMENT, WHICH INCLUDES THE SOFTWARE LICENSE AND LIMITED WARRANTY. IF YOU DO NOT AGREE WITH THESE TERMS AND CONDITIONS, YOU SHOULD PROMPTLY RETURN THE PACKAGE UNOPENED TO MIRAIBIO, INC. ("Mirai") or Mirai Distributor AND YOUR MONEY WILL BE REFUNDED.

The enclosed software is licensed, not sold, to you for use only upon the terms of this Agreement, and Mirai reserves any rights not expressly granted to you. You are responsible for the selection of the Software to achieve your intended results, and for the installation, use and results obtained from the Software. You own the media on which the Software is originally or subsequently recorded or fixed, but Mirai retains ownership of all copies of the Software itself.

LICENSE

You may:

a. Use the Software on a single machine at any given time.

b. Obtain limited numbers of Copy Protection Devices. Additional, Copy Protection Devices are provided only as a convenience of running the software.

c. In no manner engineer or reverse-engineer the copy protection hardware, or whole or part of the software.

d. Copy the software only for backup provided that you reproduce all copyright and other proprietary notices that are on the original copy of the Software provided to you. Certain Software, however, may include mechanisms to limit or inhibit copying. Such Software is marked copy protected.

e. Transfer of the Software and all rights under this Agreement to another party together with a copy of this Agreement if the other party

agrees to accept the terms and conditions of this Agreement. If you transfer the Software, you must at the same time either transfer all copies whether in printed or machine-readable form, to the same party or destroy and copies not transferred.

RESTRICTIONS

You may not use, copy, modify, or transfer the Software, or any copy, in whole or in part, except as expressly provided for in this Agreement. Any attempt to transfer any of the rights, duties or obligations hereunder except as expressly provided for in this Agreement is void. YOU MAY NOT RENT, LEASE, LOAN, RESELL FOR PROFIT, OR DISTRIBUTE.

TERM

This Agreement is effective until terminated. You may terminate it at any time by destroying the Software together with all copies in any form. This Agreement will immediately and automatically terminate without notice if you fail to comply with any term or condition of this Agreement. You agree upon termination to promptly destroy the Software together with all copies in any form.

LIMITED WARRANTY

Mirai warrants, for the period of ninety (90) days from the date of delivery of the Software to you as evidenced by a copy of your receipt, that:

(1) The Software, unless modified by you, will perform the function described in the documentation provided by Mirai. Your sole remedy under the warranty is that Mirai will undertake to correct within a reasonable period of time any marked Software Error (failure of the Software to perform the functions described in the documentation). Mirai does not warrant that the Software will meet your requirements, that operation of the Software will be uninterrupted or error-free, or that all Software Errors will be corrected.

(2) The media on which the Software is furnished will be free from defects in materials and workmanship under normal use. Mirai will, at its option, replace or refund the purchase price of the media at no charge to you, provided you return the faulty media with proof of purchase to

Mirai. Mirai will not have any responsibility to replace or refund the purchase price of the media damaged by accident, abuse or misapplication.

THE ABOVE WARRANTIES ARE EXCLUSIVE AND IN LIEU OF ALL OTHER WARRANTIES, WHETHER EXPRESS OR IMPLIED, INCLUDING THE IMPLIED WARRANTIES OF MERCHANTABILITY AND FITNESS FOR A PARTICULAR PURPOSE. NO ORAL OR WRITTEN INFORMATION OR ADVICE GIVEN BY MIRAI, ITS EMPLOYEES, DISTRIBUTORS, OR AGENTS SHALL INCREASE THE SCOPE OF THE ABOVE WARRANTIES OR CREATE ANY NEW WARRANTIES. SOME STATES DO NOT ALLOW THE EXCLUSION OF IMPLIED WARRANTIES, SO THE ABOVE EXCLUSION MAY NOT APPLY TO YOU. IN THAT EVENT, ANY IMPLIED WARRANTIES ARE LIMITED IN DURATION TO NINETY (90) DAYS FROM THE DATE OF DELIVERY OF THE SOFTWARE. THIS WARRANTY GIVES YOU SPECIFIC LEGAL RIGHTS. YOU MAY HAVE OTHER RIGHTS, WHICH VARY FROM STATE TO STATE.

LIMITATIONS OF REMEDIES

Mirai's entire liability to you and your exclusive remedy shall be the replacement of the Software media or the refund of your purchase price as set forth above. If Mirai or the Mirai's distributors are unable to deliver replacement media which is free of defects in materials and workmanship, you may terminate this Agreement by returning the Software and your money will be refunded.

REGARDLESS OF WHETHER ANY REMEDY SET FORTH HEREIN FAILS ITS ESSENTIAL PURPOSE, IN NO EVENT WILL MIRAI BE LIABLE TO YOU FOR ANY DAMAGES, INCLUDING ANY LOST PROFITS, LOST DATA OR OTHER INCIDENTAL OR CONSEQUENTIAL DAMAGES ARISING OUT OF THE USE OR INABILITY OF SUCH DAMAGES, OR FOR ANY CLAIM BY ANY OTHER PARTY.

SOME STATES DO NOT ALLOW THE LIMITATION OR EXCLUSION OR LIABILITY FOR INCIDENTAL OR CONSEQUENTIAL DAMAGES TO THE ABOVE LIMITATION OR EXCLUSION MAY NOT APPLY TO YOU.

GOVERNMENT LICENSEE/RESTRICTED RIGHTS LEGEND

If you are acquiring the Software on behalf of any unit or agency of the United States Government, the following provisions apply.

The Government acknowledges Mirai's representation that the Software and its documentation were developed at private expense and no part of them is in the public domain.

The Government acknowledges Mirai's representation that the Software is Restricted Computer Software as that term is defined in Clause 52.227-19(a) of the Federal Acquisition Regulations (FAR).

The Government acknowledges that the Software is classified as Commercial Computer Software and the Government is acquiring only restricted rights in the Software and its documentation will be as defined in Clause 52.227-19 (c)(1) and (2) of the FAR.

Manufacturer is MiraiBio, Inc., 1201 Harbor Bay Parkway, Suite 150, Alameda, CA 94502

EXPORT LAW ASSURANCES

You acknowledge and agree that the Software is subject to restrictions and controls imposed by the United States Export Administration Act ("The Act") and the regulations thereunder. You agree and certify that neither the Software nor any direct product thereof is being or will be acquired, shipped, transferred or reexported, directly or indirectly, into any country prohibited by the Act and the regulations thereunder or will be used for any purpose prohibited by the same.

GENERAL

This agreement will be governed by the laws of the State of California, except for that body of law dealing with conflicts of law.

Future updates of the Software will be available for purchase by licensees for a fee provided a registration card has been received by MiraiBio, Inc.

Should you have any questions concerning this Agreement, you may contact Mirai at http://www.miraibio.com.

You acknowledge that you have read this Agreement, understand it and agree to be bound by its terms and conditions. You further agree that it is the complete and exclusive statement of the agreement between us

which supersedes any proposal or prior agreement, oral or written, and any other communications between us in relation to the subject matter of this Agreement.

MiraiBio

MasterPlex™ GT 2.0

Genotype analysis software for multiplex data from the Luminex® system.

CONTENTS

CONTENTS

CONTENTS

CONTENTS

CHAPTER 1

WELCOME

MiraiBio MasterPlex™ GT

Welcome to the MiraiBio MasterPlex GT User Manual. MasterPlex GT software provides genotype analysis of results (.csv) from the Luminex® system.

1.1 About This Manual

This manual explains how to use the MasterPlex GT software to:

- import results files (.csv) from the Luminex system
- set allele calling parameters
- compute allele, genotype, or haplotype frequencies
- sort samples by name, expression level, or haplotype
- apply cluster analysis to the MFI data or haplotype
- generate genotype reports

What's New in MasterPlex GT 2.0

New features in MasterPlex GT 2.0 software enable you to:

- perform HLA typing using a lookup table
- merge results from different bead sets for the same sample (allows you to view results from more than 100 different bead sets per sample in the Typing table)
- merge results in the Allele Call table
- automatically launch plug-ins when MasterPlex GT starts

Conventions Used in This Manual

This manual describes the steps required to perform the various tasks associated with the MasterPlex GT software. The manual uses a step format to explain the various tasks associated with MasterPlex GT. The symbol ⇒ may follow a step instruction. It indicates the software response to the action performed by the user.

Screen Captures

Screen captures may accompany the step instructions for further illustration. The screen captures in this manual may not exactly match those displayed on your screen.

1.2
Technical Support

You can contact MiraiBio Technical support at:

MiraiBio, Inc.
1201 Harbor Bay Parkway
Suite 150
Alameda, CA 94502
USA
Tel: +1 (510) 337-2000
Toll Free: +1 (800) 624-6176
Fax: +1 (510) 337-2099
E-mail: gene@miraibio.com

www.miraibio.com

CHAPTER 2

INSTALLING MASTERPLEX GT

This chapter explains the minimum hardware and software requirements needed to install and use MasterPlex™ GT. It provides installation instructions for a computer connected to the Luminex® system.

2.1 Requirements

For optimum performance, MasterPlex GT requires hardware and software that meet or exceed the following specifications. It is also strongly recommended that you use the Luminex XY platform.

Hardware Requirements

Platform	IBM PC compatible
Memory (RAM)	64 MB RAM or higher for Windows® 98SE, 128MB or higher for Windows 2000/XP
Storage space (HDD)	20 MB available space for the installation
Input devices	Keyboard and mouse or any other pointing device
Video RAM	4MB or higher
Monitor resolution	SVGA (1024x768) pixels or higher
Monitor color	16-bit color (high color) or higher
CD-ROM drive	Required for CD media version. Not applicable for download version.

Software Requirements

Operating system	Microsoft Windows 98SE/2000/XP only

2.2 Installing MasterPlex GT

1. Insert the MasterPlex GT CD-ROM in the workstation computer and double-click MasterPlex GT.exe.

⇒ The installation process begins and the InstallShield Wizard appears (Figure 2.1).

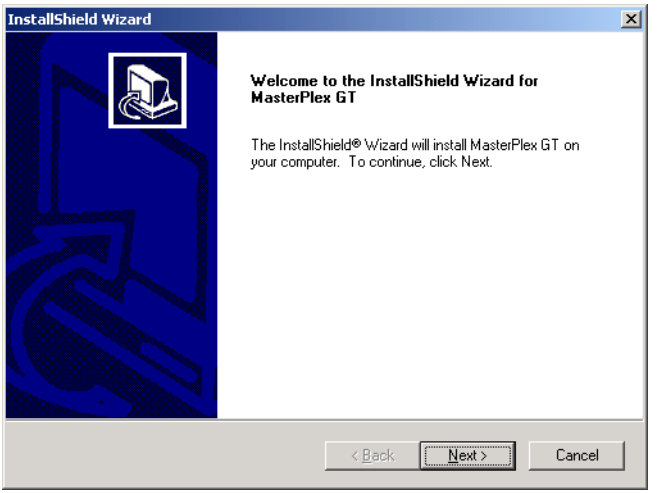


Figure 2.1 InstallShield Wizard

2. To continue the installation, click **Next**.
   ⇒ The Choose Destination Location window appears (Figure 2.2).

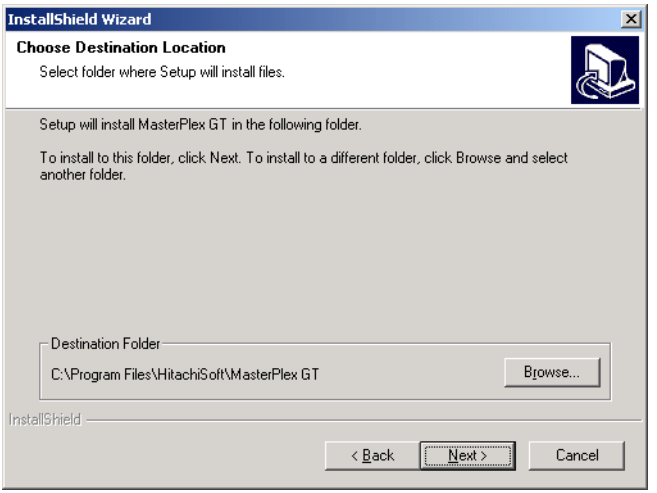


Figure 2.2 Install Shield Wizard, Choose Destination Location

3. Confirm the default destination folder or click **Browse** to specify a different folder.

   The destination folder is where the program files will be installed.

4. Click **Next**.

   ⇒ The program is installed. When the installation is complete, the InstallShield Wizard Complete window appears (Figure 2.3).

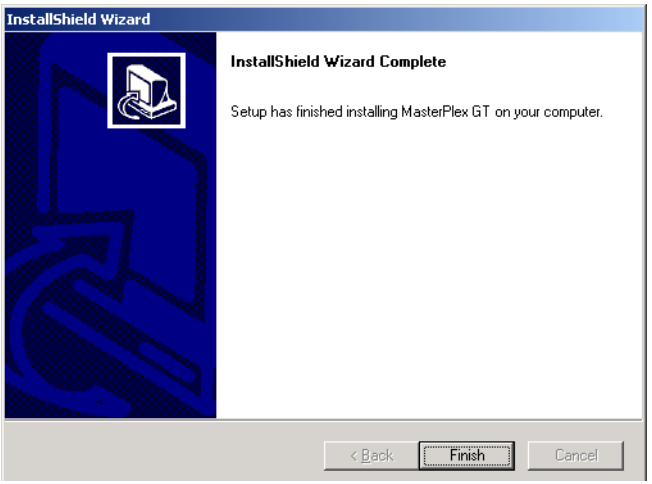


Figure 2.3 InstallShield Wizard Complete window

5. Click **Finish**.

2.3 Installing a License

1. Double-click the MasterPlex™ GT icon on the workstation desktop.

   ⇒ The License Information dialog box appears (Figure 2.4).

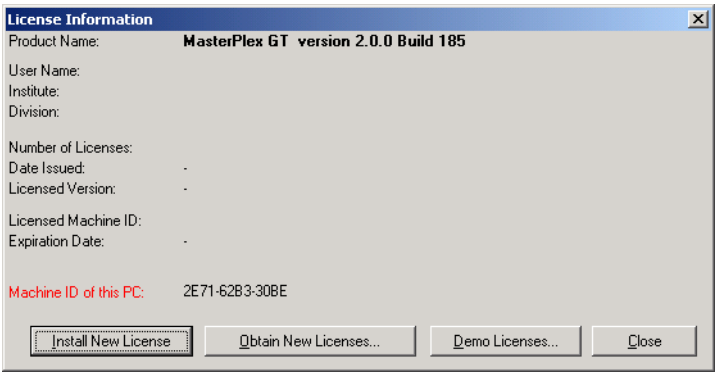


Figure 2.4 License Information dialog box

2. To view instructions on how to obtain a license (.lic), click **Obtain New Licenses**.
3. After you have obtained a license, click **Install New License**.
   ⇒ The Open dialog box appears.
4. Use the Open dialog box to locate the license (.lic) and double-click the file.
   ⇒ The license is installed.
5. To view the license information, select **Help → View License Info** from the menu bar.
   ⇒ The license information is displayed (Figure 2.5).

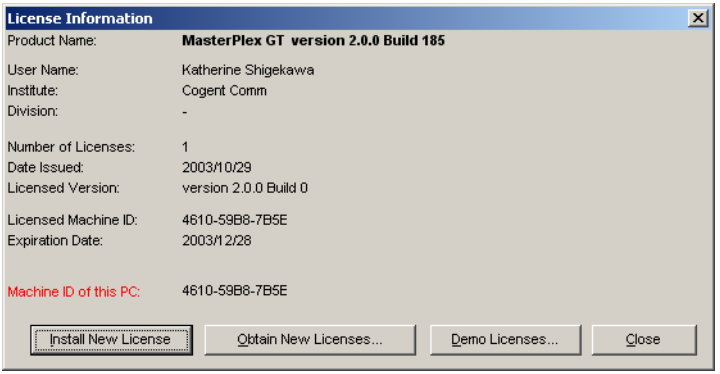


Figure 2.5 License information

CHAPTER 3

BEFORE YOU BEGIN

This chapter explains the MasterPlex™ GT bead naming conventions. It also provides an overview of genotype analysis using the MasterPlex GT software.

3.1 Bead Name Conventions

The MasterPlex GT software organizes results data (.csv) by bead and sample name in the Typing table (Figure 3.1). (See *The Typing Table* on page 7.1 for more information.) Further, the software can combine or *merge* results that are derived from identically named bead sets or merge the results from different bead sets that probe the same sample. The merged data are displayed in one Typing table, enabling you to analyze and compare samples across experiments or view the results from more than 100 different bead sets per sample. (See *Merging Results* on page 4.11 for more information.)

The MasterPlex bead naming convention includes a group and allele name Table 3.1. To follow this naming convention, in the Luminex® data collection software specify:

- a prefix that identifies the gene region that the probe interrogates. For example, the prefix can identify a locus, intron or exon, or other marker (see Table 3.1 and Table 3.2).
- an allele name that identifies the variation at the gene region that the probe interrogates. For example, the name can identify an allele or a SNP base (see Table 3.1 and Table 3.2).

The prefix is the default group name that the MasterPlex GT software automatically assigns to each bead. The Typing table sorts the bead names by group (locus) name, then by allele name Figure 3.1. This enables you to plan a bead naming strategy that optimizes the Typing table view for your needs. Table 3.1 summarizes the MasterPlex GT bead naming conventions.

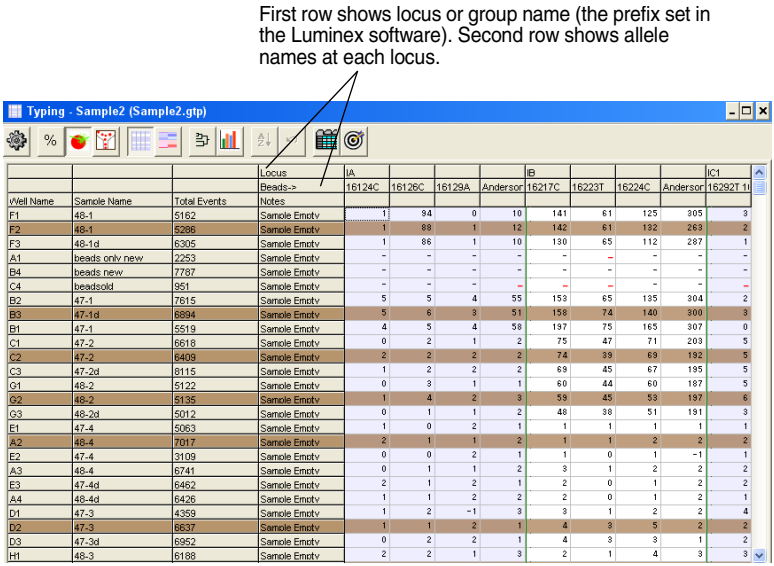
Table 3.1 MasterPlex™ GT bead naming conventions

Name Component	Specified...	Identifies the...
Prefix	By the user in the Luminex® software. The prefix can be edited in MasterPlex GT.	Gene region or marker that the probe interrogates.
Group (locus) name	Automatically by the MasterPlex GT software. (The prefix is the default group name.) The group name can be edited in MasterPlex GT.	Gene region or marker that the probe interrogates.
Allele name	By the user in the Luminex software. The allele name can be edited in the MasterPlex GT software.	Variation that the probe interrogates.

Table 3.2 shows example names for the beads that interrogate human mitochondrial DNA at Hypervariable Region IA. Figure 3.1 shows how the Typing table organizes the bead names by locus (group) and allele name. If the bead names do not follow the MasterPlex GT naming convention, the Typing table displays them in the order that the data were collected ((Figure 3.2)).

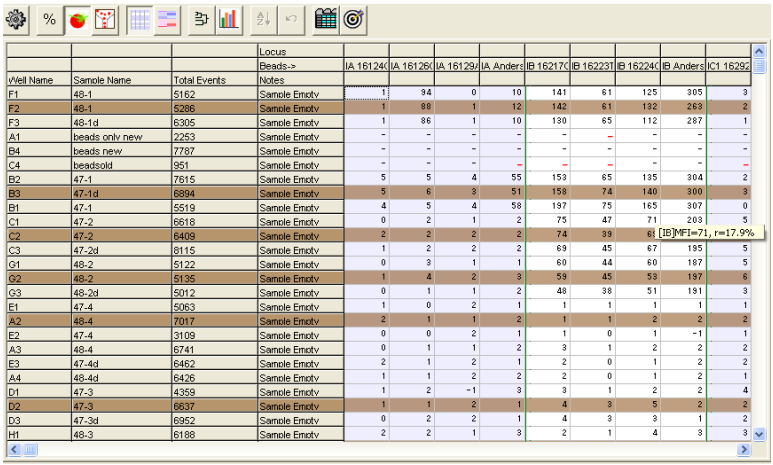
Table 3.2 Example group and allele names for beads that interrogate human mitochondrial DNA at Hypervariable Region IA

Group (Prefix) Name	Allele Name	In the Luminex software (Parameter Settings), enter...
IA	16124C	IA_16124C or IA 16124C
IA	16126C	IA_16126C or IA 16126C
IA	16129A	IA_16129A or IA 16129A
IA	Anderson	IA_Anderson or IA Anderson



			Locus	IA				IB				IC1
			Beads->	16124C	16126C	16129A	Anderson	16217C	16223T	16224C	Anderson	16292T 1
Well Name	Sample Name	Total Events	Notes									
F1	48-1	5162	Sample Empty	1	94	0	10	141	61	125	305	3
F2	48-1	5286	Sample Empty	1	88	1	12	142	61	132	263	2
F3	48-1d	6305	Sample Empty	1	86	1	10	130	65	112	287	1
A1	beads only new	2253	Sample Empty	-	-	-	-	-	-	-	-	-
B4	beads new	7787	Sample Empty	-	-	-	-	-	-	-	-	-
C4	beadsold	951	Sample Empty	-	-	-	-	-	-	-	-	-
B2	47-1	7615	Sample Empty	5	5	4	55	153	65	135	304	2
B3	47-1d	6894	Sample Empty	5	6	3	51	158	74	140	300	3
B1	47-1	5519	Sample Empty	4	5	4	58	197	75	165	307	0
C1	47-2	6618	Sample Empty	0	2	1	2	75	47	71	203	5
C2	47-2	6409	Sample Empty	2	2	2	2	74	39	69	192	5
C3	47-2d	8115	Sample Empty	1	2	2	2	69	45	67	195	5
G1	48-2	5122	Sample Empty	0	3	1	1	60	44	60	187	5
G2	48-2	5135	Sample Empty	1	4	2	3	59	45	53	197	6
G3	48-2d	5012	Sample Empty	0	1	1	2	48	38	51	191	3
E1	47-4	5063	Sample Empty	1	0	2	1	1	1	1	1	1
A2	48-4	7017	Sample Empty	2	1	1	2	1	1	2	2	2
E2	47-4	3109	Sample Empty	0	0	2	1	1	0	1	-1	1
A3	48-4	6741	Sample Empty	0	1	1	2	3	1	2	2	2
E3	47-4d	6462	Sample Empty	2	1	2	1	2	0	1	2	2
A4	48-4d	6426	Sample Empty	1	1	2	2	2	0	1	2	1
D1	47-3	4359	Sample Empty	1	2	-1	3	3	1	2	2	4
D2	47-3	8637	Sample Empty	1	1	2	1	4	3	5	2	2
D3	47-3d	6952	Sample Empty	0	2	2	1	4	3	3	1	2
H1	48-3	6188	Sample Empty	2	2	1	3	2	1	4	3	3

Figure 3.1 Typing table
Bead names sorted by prefix and allele name.



			Locus									
			Beads->	IA 16124(	IA 16126(	IA 16129/	IA Anders	IB 16217(	IB 16223T	IB 16224(	IB Anders	IC1 1629
Well Name	Sample Name	Total Events	Notes									
F1	48-1	5162	Sample Empty	1	94	0	10	141	61	125	305	3
F2	48-1	5286	Sample Empty	1	88	1	12	142	61	132	263	2
F3	48-1d	6305	Sample Empty	1	86	1	10	130	65	112	287	1
A1	beads only new	2253	Sample Empty	-	-	-	-	-	-	-	-	-
B4	beads new	7787	Sample Empty	-	-	-	-	-	-	-	-	-
C4	beadsold	951	Sample Empty	-	-	-	-	-	-	-	-	-
B2	47-1	7615	Sample Empty	5	5	4	55	153	65	135	304	2
B3	47-1d	6894	Sample Empty	5	6	3	51	158	74	140	300	3
B1	47-1	5519	Sample Empty	4	5	4	58	197	75	165	307	0
C1	47-2	6618	Sample Empty	0	2	1	2	75	47	71	203	5
C2	47-2	6409	Sample Empty	2	2	2	2	74	39	69	192	5
C3	47-2d	8115	Sample Empty	1	2	2	2	69	45	67	195	5
G1	48-2	5122	Sample Empty	0	3	1	1	60	44	60	187	5
G2	48-2	5135	Sample Empty	1	4	2	3	59	45	53	197	6
G3	48-2d	5012	Sample Empty	0	1	1	2	48	38	51	191	3
E1	47-4	5063	Sample Empty	1	0	2	1	1	1	1	1	1
A2	48-4	7017	Sample Empty	2	1	1	2	1	1	2	2	2
E2	47-4	3109	Sample Empty	0	0	2	1	1	0	1	-1	1
A3	48-4	6741	Sample Empty	0	1	1	2	3	1	2	2	2
E3	47-4d	6462	Sample Empty	2	1	2	1	2	0	1	2	2
A4	48-4d	6426	Sample Empty	1	1	2	2	2	0	1	2	1
D1	47-3	4359	Sample Empty	1	2	-1	3	3	1	2	2	4
D2	47-3	8637	Sample Empty	1	1	2	1	4	3	5	2	2
D3	47-3d	6952	Sample Empty	0	2	2	1	4	3	3	1	2
H1	48-3	6188	Sample Empty	2	2	1	3	2	1	4	3	3

Figure 3.2 Typing table
Bead names displayed in the order that the data were collected.

Choosing a Bead Name Option

You can choose between the two bead naming options that determine how the Typing table displays the results data.

1. Select **Option → Set Application Options** from the menu bar.
   ⇒ The Application Options dialog box opens (Figure 3.3).

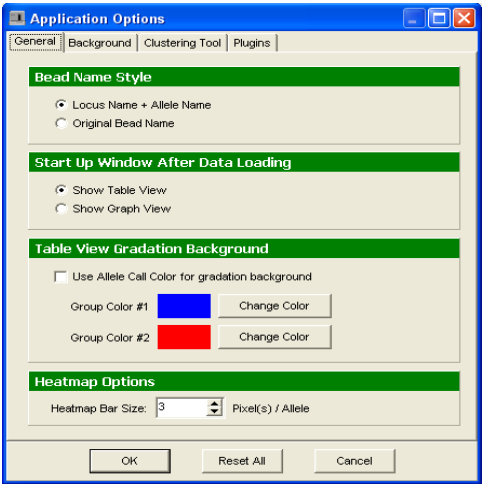


Figure 3.3 Application Options dialog box, General tab

2. To organize bead names in the Typing table by group and allele name (Figure 3.1), choose the **Locus Name + Allele Name** option.
3. To organize bead names in the Typing table in the order that the data were collected (Table 3.2), choose the **Original Bead Name** option.
4. Click **OK**.

Editing the Bead Name

The prefix set in the Luminex® software is the default locus (group) name in the MasterPlex™ GT software (Figure 3.1). You can edit the prefix, group, or allele name for a bead in the Parameter Settings dialog box or the Typing table.

To rename a bead in the Parameter Settings dialog box:

1. Click the **Parameter Setting** button  .
   ⇒ The Parameter Setting dialog box appears (Figure 3.4).

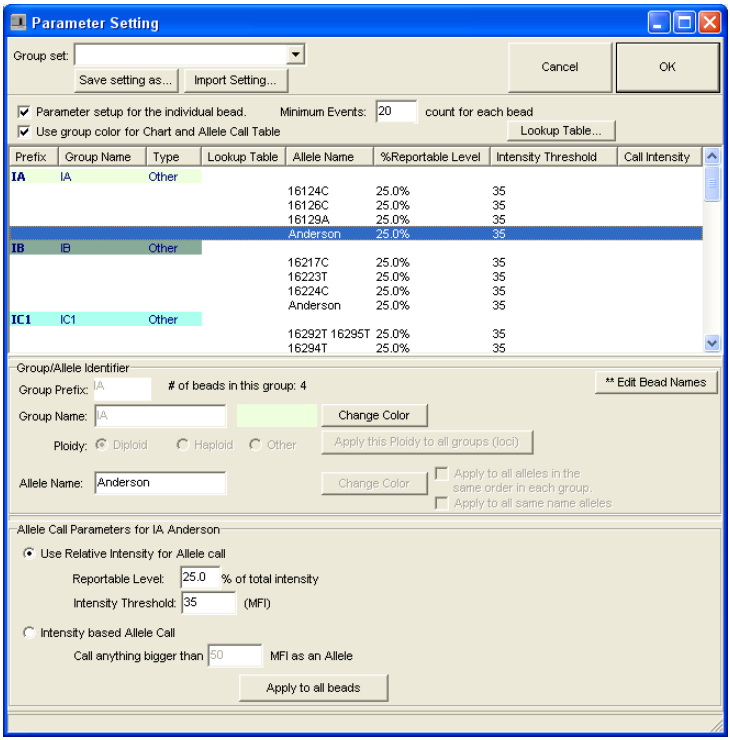


Figure 3.4 Parameter Setting dialog box

2. Click **Edit Bead Names**.
   ⇒ The Edit Bead Names dialog box opens (Figure 3.5).

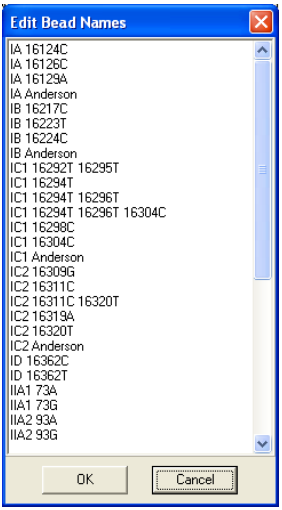


Figure 3.5 Edit Bead Names dialog box

3. Select the bead name you want to edit and enter a new name.
4. Click **OK** when you finish editing the names.

   ⇒ The new bead name (prefix, group, and allele name) is displayed in the Parameter Setting dialog box, Typing table, graph view, and statistics table.

To rename a bead in the Typing table:

1. Right-click a locus or allele name in the Typing table.
2. Click **Edit Bead Name** in the pop-up menu that appears (Figure 3.6).

   ⇒ The Change Bead Name dialog box appears (Figure 3.7).
3. Edit the bead name and click **OK**.

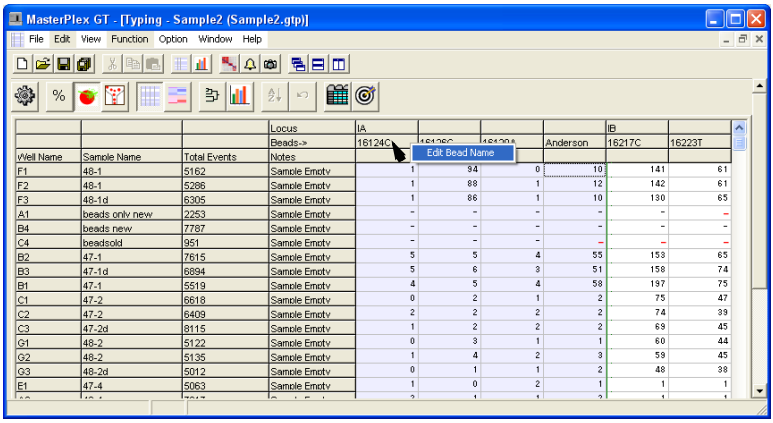


Figure 3.6 Typing table
Right-click a bead name to edit the name.

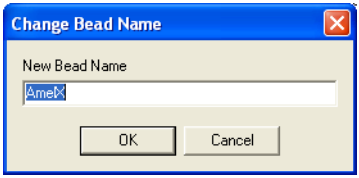


Figure 3.7 Change Bead Name dialog box

Editing the Group or Allele Name Only

You can also edit just the group or allele name in the Parameter Settings dialog box.

1. In the Parameter Settings dialog box, select the group or allele name you want to edit.

   ⇒ The Group Name or Allele Name box below displays the selected name component (Figure 3.8).

   For example, the allele name 16217C is selected in Figure 3.8.

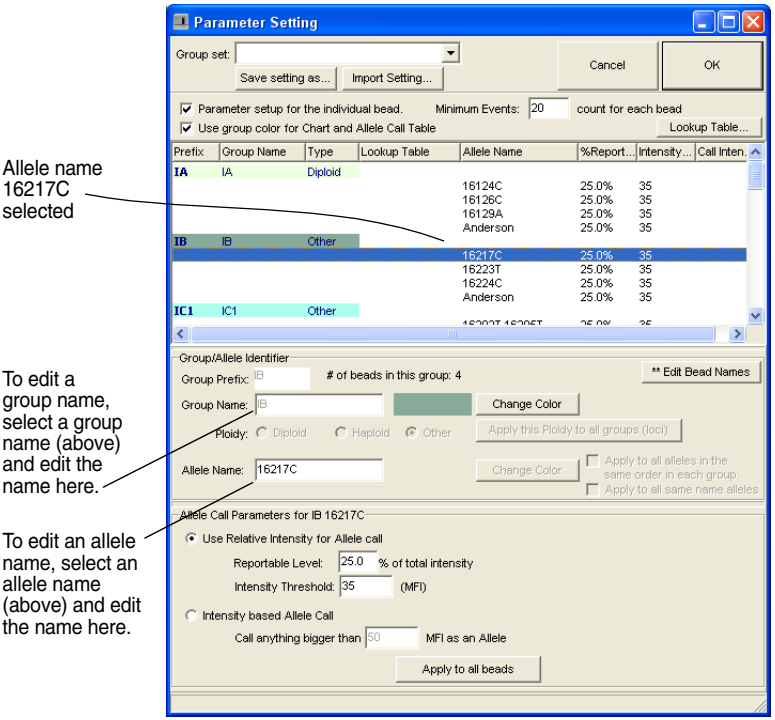


Figure 3.8 Parameter Setting dialog box

2. Edit the name in the Group Name or Allele Name box.
3. Click **Apply**.
   ⇒ The Parameter Settings dialog box displays the new name.

3.2
Overview of MasterPlex GT Analysis

This section provides an overview of MasterPlex™ GT genotype analysis. An analysis workflow includes the following steps:

1. Open the results files (.csv) of interest and view the Project Manager and Project Window (Figure 3.10). A separate Project Window opens for each results file and displays the intensity data in the Typing table (Figure 3.10).

2. If necessary, merge results that you want to combine and analyze in one Typing table. You can merge samples and compare results across experiments (sample merge) or combine results from different bead sets that probe the same sample (layer merge).
3. Set negative controls.
4. Confirm the defaults or choose new allele calling parameters (Figure 3.9).
5. View the genotyping results in the table or graph format (see Table 3.3).
6. Save the results to a project (.gtp) that includes analysis parameter settings, graphs, dendrogram, and user-selected samples.

Table 3.3 MasterPlex GT table and graph formats

Format	Displays...
Typing table Figure 3.10	Background-adjusted median fluorescence intensity (MFI), relative intensity (RI), bead count, or allele frequency data.
Heat Map Figure 3.12	A color-coded map bead MFI data for each sample.
Allele Call window Figure 3.13	Four tables: allele calls, allele frequency, genotype frequency, and haplotype frequency.
Homology table Figure 3.14	The homology score between sample genotypes.
Homology chart Figure 3.15	A plot of the correlation coefficients between sample genotypes.
Multi Compare bar graph Figure 3.16	A bar graph of the background-adjusted MFI or RI values for a user-selected sample.
Depth Bar graph Figure 3.16	A composite bar graph of the background-adjusted MFI or RI values for user-selected samples.
Sample scatter graph Figure 3.18	A scatter plot of the background adjusted MFI data for a user-selected pair of samples. Each graph point represents an allele.
Allele scatter graph Figure 3.19	A scatter plot of the background adjusted MFI data for a user-selected pair of alleles. Each graph point represents a sample.
Dendrogram Figure 3.20	A diagram of the sample cluster analysis results.

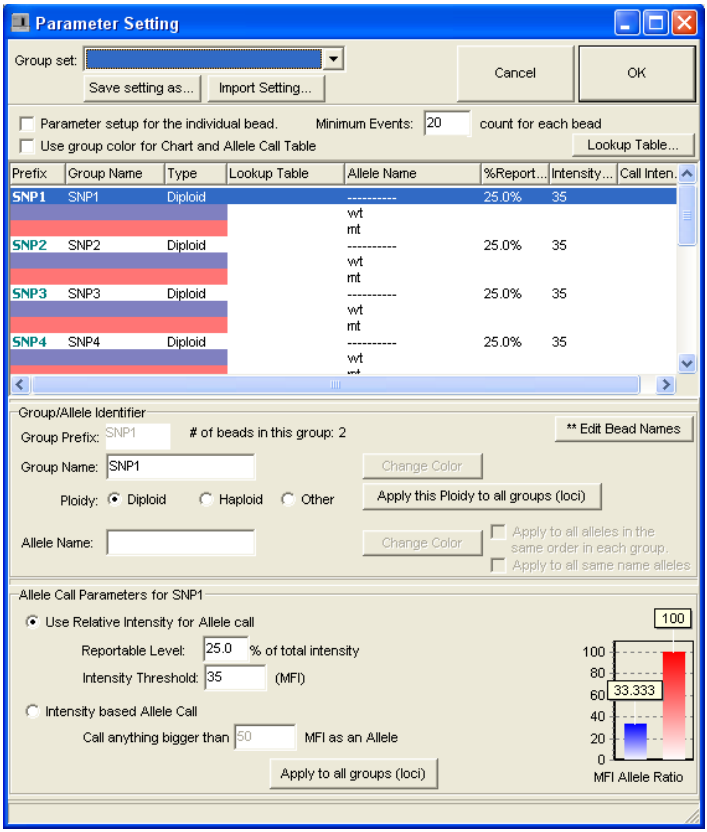
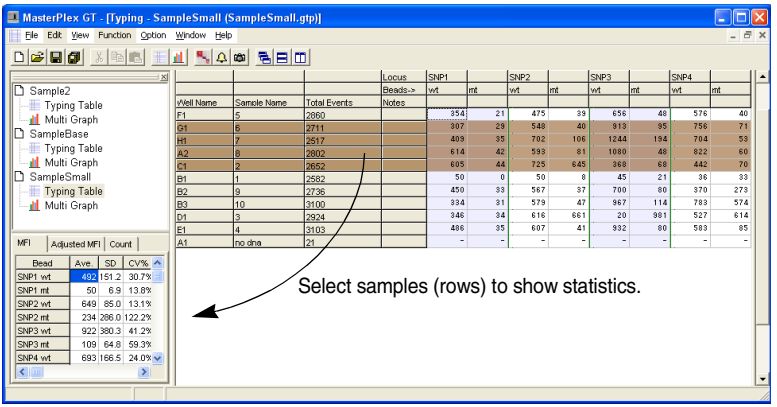


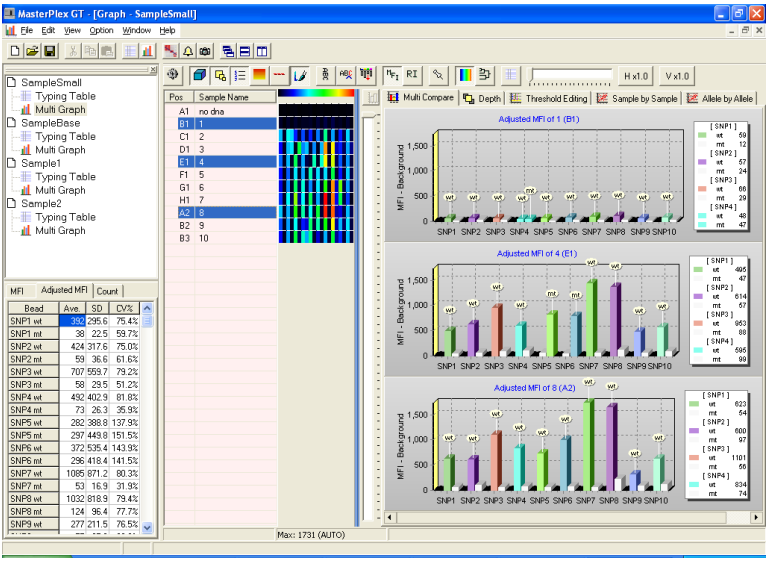
Figure 3.9 Parameter Setting dialog box
Allele calling parameters and options.



Project Manager includes file tree (top) and statistics table (bottom).

Typing table in the Project Window.

Figure 3.10 Project Manager and Project Window
Typing table displayed in the Project Window.



Project Manager includes file tree (top) and Statistics table (bottom)

Project Window displaying the Heat map and Multi Compare graph

Figure 3.11 Project Manager and Project Window
Multi Compare graph in the Project Window.

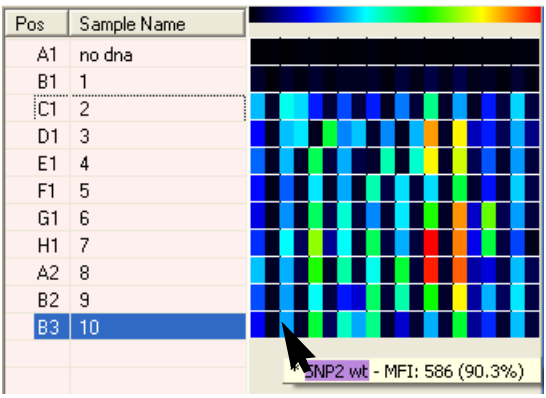


Figure 3.12 Heat map

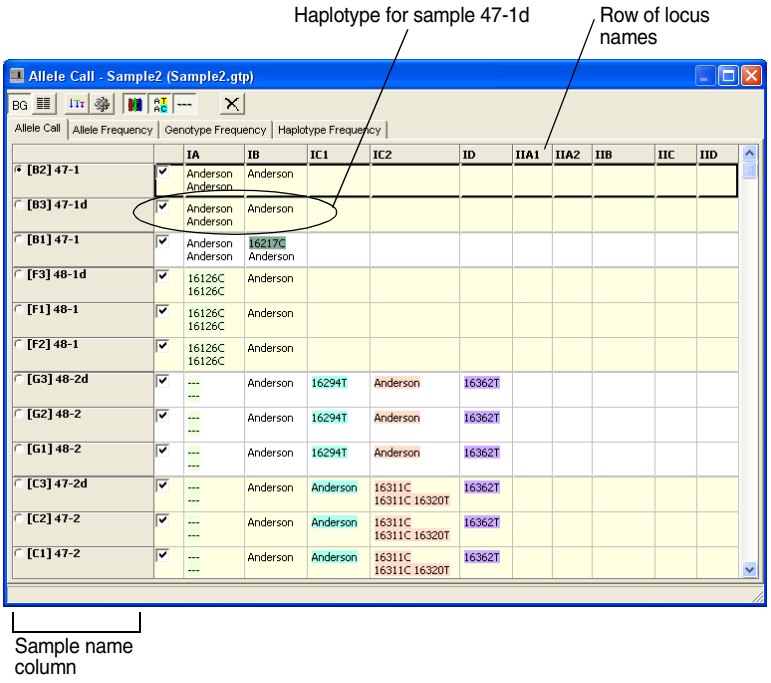


Figure 3.13 Allele Call table displays the alleles called for each sample

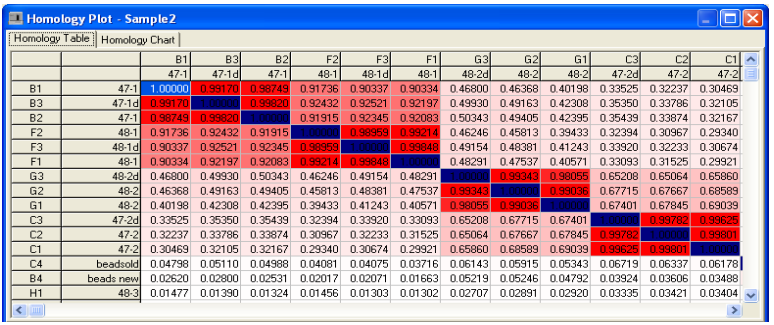


Figure 3.14 Homology table
Correlation coefficient between sample genotypes are displayed.

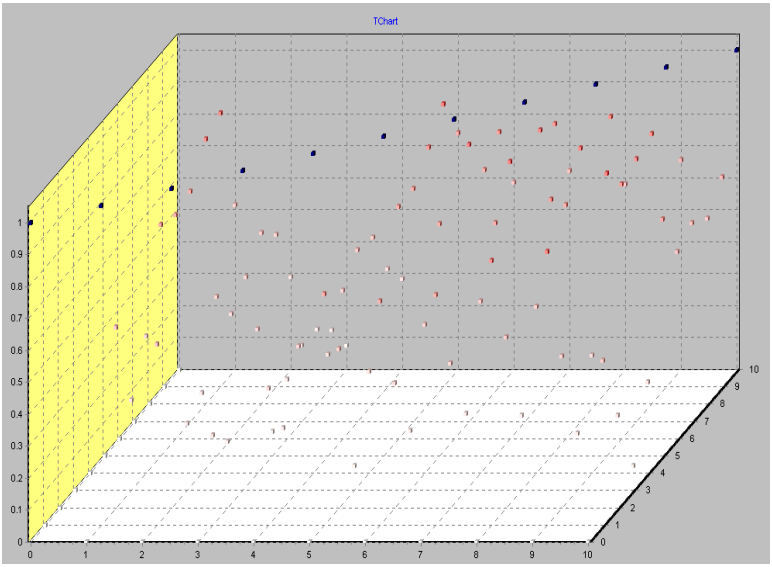


Figure 3.15 Homology chart
The correlation coefficients between sample genotypes are plotted.

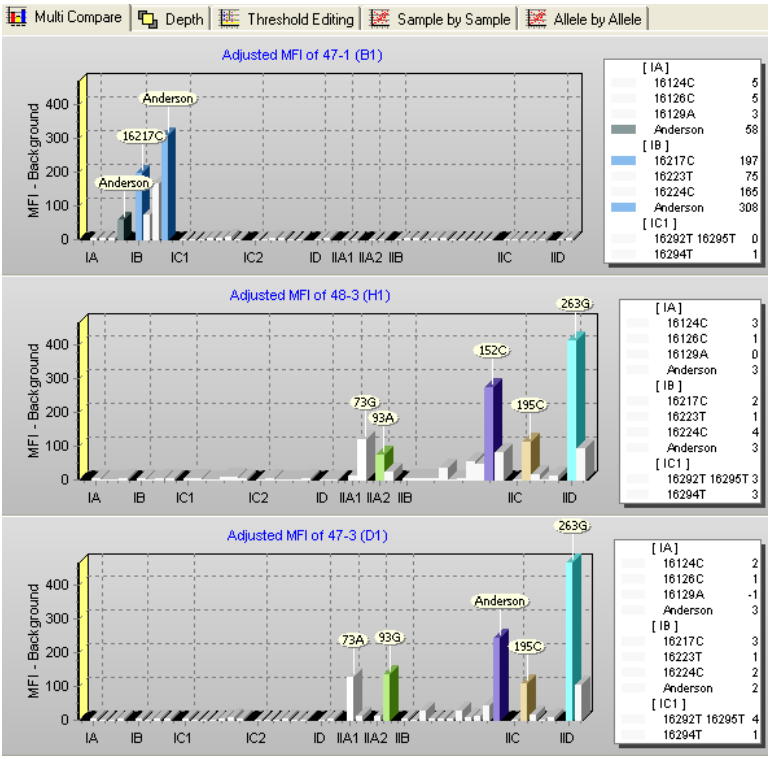


Figure 3.16 Multi Compare bar graphs
The graphs display allele MFI or RI values for user-selected samples.

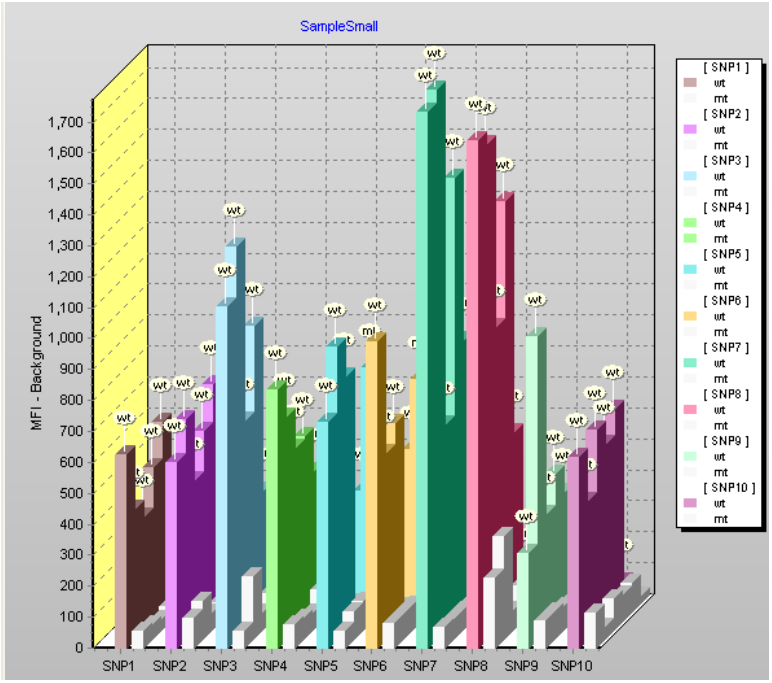


Figure 3.17 Depth bar graph
Each bar graph shows MFI or RI values for user-selected samples.

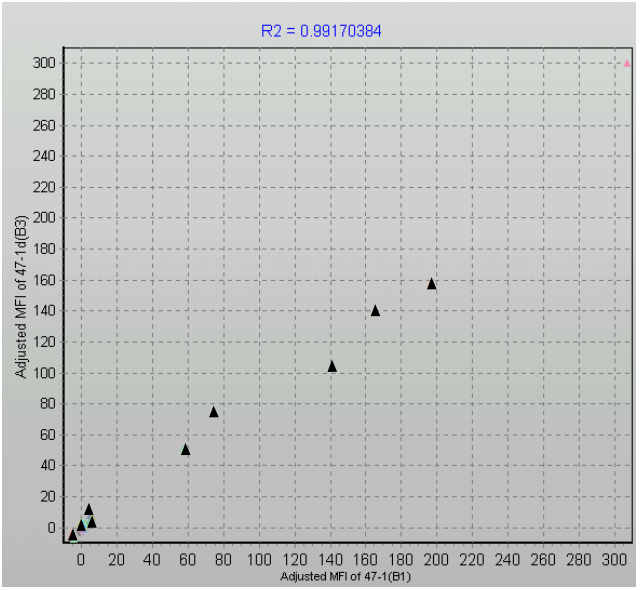


Figure 3.18 Sample by Sample scatter graph
Each point represents an allele.

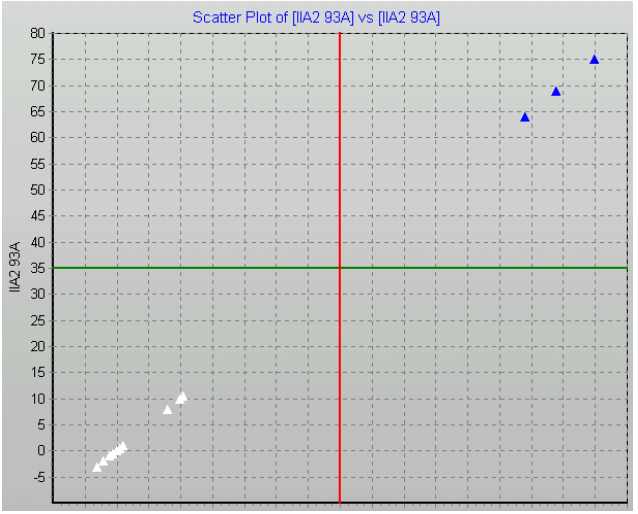


Figure 3.19 Allele by Allele scatter graph
Each point represents a sample.

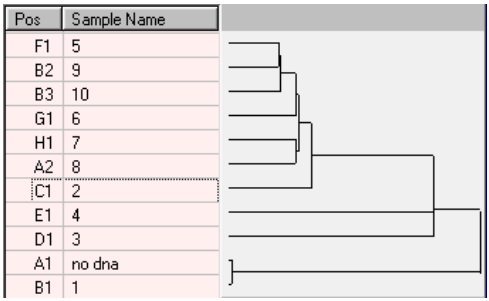


Figure 3.20 Dendrogram
Example cluster analysis results.

This chapter explains how to open Luminex results (.csv). It also explains how to combine or merge results. Results can be merged two different ways: a sample merge combines results across experiments that use identically named bead sets, a layer merge combines the results from different bead sets that probe the same sample.

4.1 The Project Manager and Project Window

The Project Manager and Project Window appear when you open Luminex results (.csv). In MasterPlex GT, the results are called projects and include the:

- the Typing table
- graphs or dendrogram created in the Multi Graph view
- parameter settings
- user-selected samples

A project can be saved (.gtp) for future sessions.

Project Manager (Figure 4.1)	Displays a file tree of open results (projects) and the Statistics table.
Project Window (Figure 4.2):	Displays the Typing table or Multi Graph view of the results data.

The Project Manager is anchored or *docked* to the Project Window (Figure 4.3) You can undock the Project Manager from the Project Window. This enables you to change the position of the Project Manager relative to the Project Window.

More than one project may be open at a time and each is displayed in a separate Project Window. Table 4.1 on page 4.4 shows the options available to you for displaying the Project Manager and Project Windows.

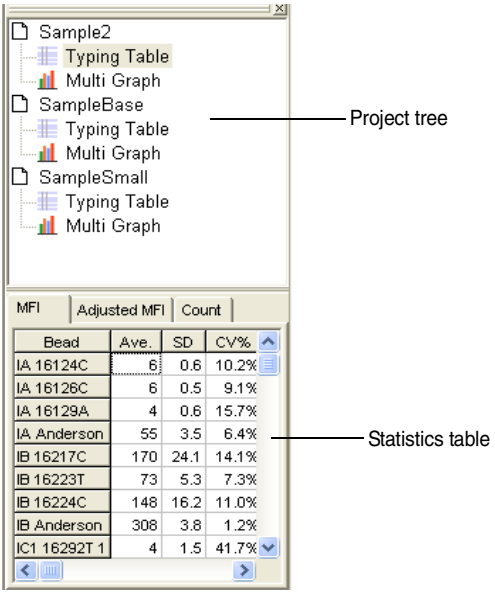
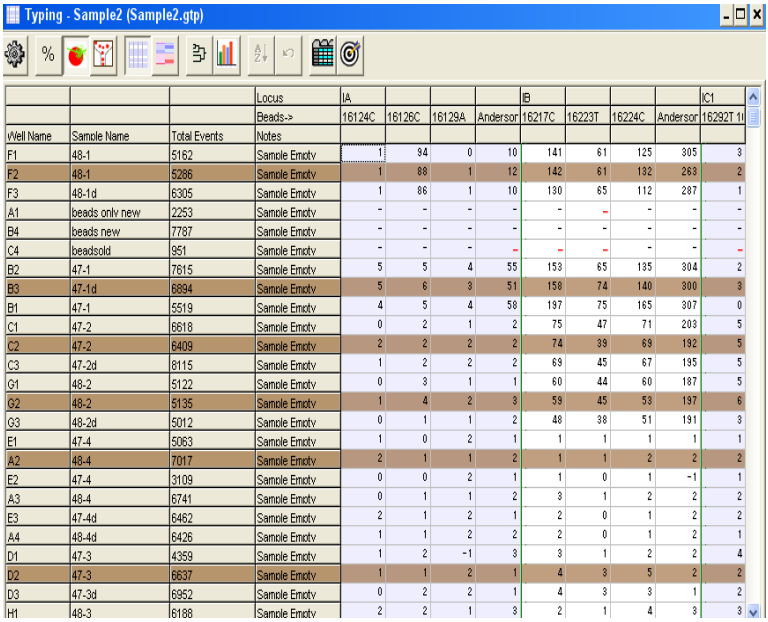


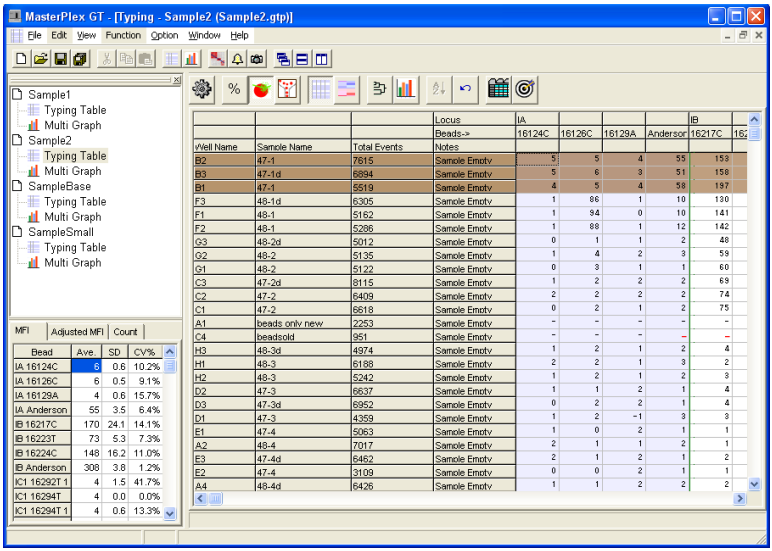
Figure 4.1 Project Manager

The Project Manager includes two components: a tree of open results and a statistics table for samples selected in the Typing table.



			Locus	IA				IB				IC1
			Beads->	16124C	16126C	16129A	Anderson	16217C	16223T	16224C	Anderson	16292T 1
Well Name	Sample Name	Total Events	Notes									
F1	48-1	5162	Sample Empty	1	94	0	10	141	61	125	305	3
F2	48-1	5286	Sample Empty	1	88	1	12	142	61	132	263	2
F3	48-1d	6305	Sample Empty	1	86	1	10	130	65	112	287	1
A1	beads only new	2253	Sample Empty	-	-	-	-	-	-	-	-	-
B4	beads new	7787	Sample Empty	-	-	-	-	-	-	-	-	-
C4	beadsold	951	Sample Empty	-	-	-	-	-	-	-	-	-
B2	47-1	7615	Sample Empty	5	5	4	55	153	65	135	304	2
B3	47-1d	6894	Sample Empty	5	6	3	51	158	74	140	300	3
B1	47-1	5519	Sample Empty	4	5	4	58	197	75	165	307	0
C1	47-2	6618	Sample Empty	0	2	1	2	75	47	71	203	5
C2	47-2	6409	Sample Empty	2	2	2	2	74	39	69	192	5
C3	47-2d	8115	Sample Empty	1	2	2	2	69	45	67	195	5
G1	48-2	5122	Sample Empty	0	3	1	1	60	44	60	187	5
G2	48-2	5135	Sample Empty	1	4	2	3	59	45	53	197	6
G3	48-2d	5012	Sample Empty	0	1	1	2	48	38	51	191	3
E1	47-4	5063	Sample Empty	1	0	2	1	1	1	1	1	1
A2	48-4	7017	Sample Empty	2	1	1	2	1	1	2	2	2
E2	47-4	3109	Sample Empty	0	0	2	1	1	0	1	-1	1
A3	48-4	6741	Sample Empty	0	1	1	2	3	1	2	2	2
E3	47-4d	6462	Sample Empty	2	1	2	1	2	0	1	2	2
A4	48-4d	6426	Sample Empty	1	1	2	2	2	0	1	2	1
D1	47-3	4359	Sample Empty	1	2	-1	3	3	1	2	2	4
D2	47-3	6637	Sample Empty	1	1	2	1	4	3	5	2	2
D3	47-3d	6952	Sample Empty	0	2	2	1	4	3	3	1	2
H1	48-3	6188	Sample Empty	2	2	1	3	2	1	4	3	3

Figure 4.2 Project Window, Typing table view



			Locus	IA				IB	
			Beads->	16124C	16126C	16129A	Anderson	16217C	16
Well Name	Sample Name	Total Events	Notes						
B2	47-1	7615	Sample Empty	5	5	4	55	153	
B3	47-1d	6894	Sample Empty	5	6	3	51	158	
B1	47-1	5519	Sample Empty	4	5	4	58	197	
F3	48-1d	6305	Sample Empty	1	86	1	10	130	
F1	48-1	5162	Sample Empty	1	94	0	10	141	
F2	48-1	5286	Sample Empty	1	88	1	12	142	
G3	48-2d	5012	Sample Empty	0	1	1	2	48	
G2	48-2	5135	Sample Empty	1	4	2	3	59	
G1	48-2	5122	Sample Empty	0	3	1	1	60	
C3	47-2d	8115	Sample Empty	1	2	2	2	69	
C2	47-2	6409	Sample Empty	2	2	2	2	74	
C1	47-2	6618	Sample Empty	0	2	1	2	75	
A1	beads only new	2253	Sample Empty	-	-	-	-	-	
C4	beadsold	951	Sample Empty	-	-	-	-	-	
H3	48-3d	4974	Sample Empty	1	2	1	2	4	
H1	48-3	6188	Sample Empty	2	2	1	3	2	
H2	48-3	5242	Sample Empty	1	2	1	2	3	
D2	47-3	6637	Sample Empty	1	1	2	1	4	
D3	47-3d	6952	Sample Empty	0	2	2	1	4	
D1	47-3	4359	Sample Empty	1	2	-1	3	3	
E1	47-4	5063	Sample Empty	1	0	2	1	1	
A2	48-4	7017	Sample Empty	2	1	1	2	1	
E3	47-4d	6462	Sample Empty	2	1	2	1	2	
E2	47-4	3109	Sample Empty	0	0	2	1	1	
A4	48-4d	6426	Sample Empty	1	1	2	2	2	

Figure 4.3 Project Manager docked to Project Window

Table 4.1 Project manager and project window display options

Display Option	Command
Undock the Project Manager from Project windows	Select **View → Dock/Undock Project Manager** from the menu bar or double-click the Project Manager title bar.
Hide the Project Manager	Click the Project Manager **Close** button or select **View → Show/Hide Project Manager** from the menu bar.
Tile Project windows in cascade	Click the button or select **Windows → Cascade** from the menu bar.
Tile Project windows horizontally	Click the button or select **Windows → Tile Horizontally** from the menu bar.
Tile Project windows vertically	Click the button or select **Windows → Tile Vertically** from the menu bar.
Minimize all project windows	**Windows → Minimize All** from the menu bar.
Organize all minimized project windows	**Windows → Arrange All** from the menu bar.

Viewing Data

The Project Window shows two views of the results data:

- Typing table (Figure 4.4) (See *The Typing Table* on page 7.1 for more information.)
- Multi Graph view (Figure 4.5) that displays different graphical formats (See *The Multi Graph View* on page 8.1 for more information.)

There are two ways to show the Typing table. To view the Typing table, do either of the following:

- Click the Typing table button  under the file of interest in the Project manager
- Make a selection from the **Window** menu in the menu bar

There are two ways to show the MultiGraph view. To display the Multi Graph view, do either of the following:

- Click the Multi Graph button  under the project of interest in the Project Manager
- Make a selection from the **Window** menu in the menu bar

NOTE: The graph view is blank until samples are selected for graphing.

- To show or hide the Heat map, click the Heat Map toolbar button  .

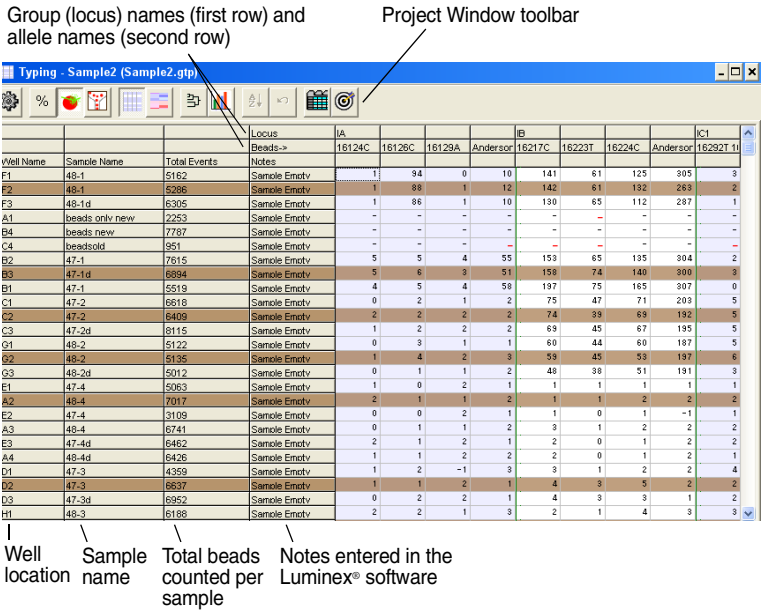


Figure 4.4 Project Window, Typing table displaying MFI data

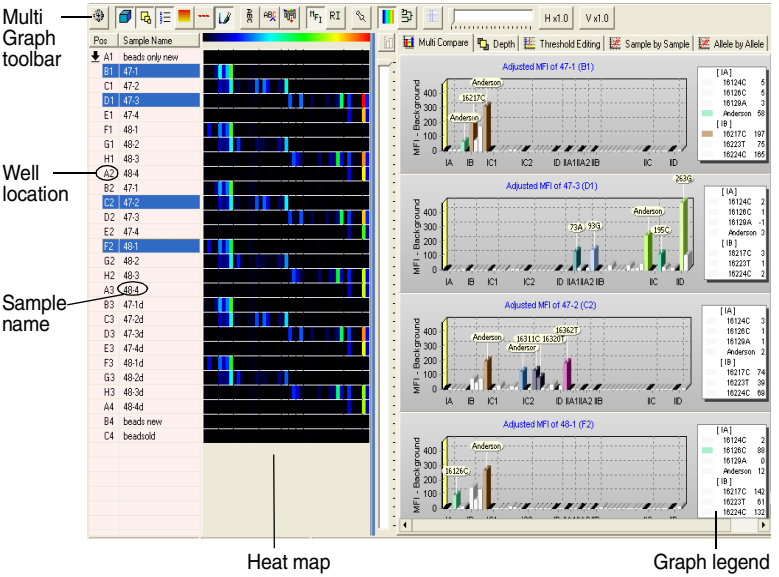


Figure 4.5 Project Window
Multi Graph view shows the Multi Compare graphs for the selected samples.

Viewing Project Information

1. To view project information, right-click the project name in the Project Manager and select **View File Info** from the shortcut menu.
   ⇒ The Project information is displayed (Figure 4.6).

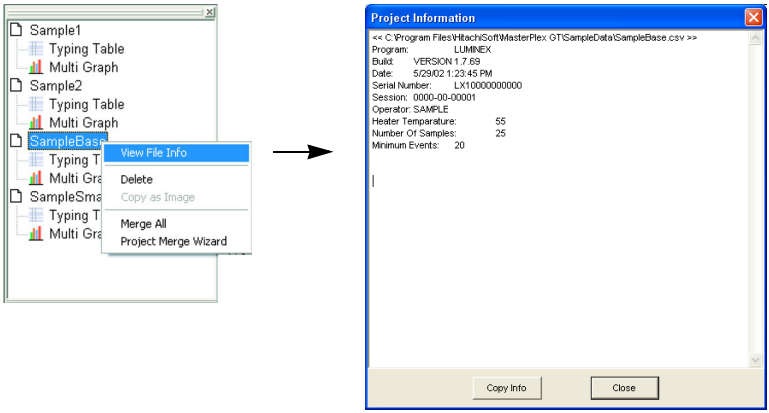


Figure 4.6 File information

2. To copy the file information to the system clipboard, click **Copy Info**.

Removing Projects from the Project Manager

To remove a project from the Project Manager, right-click the project name and select **Delete** from the shortcut menu.

NOTE: This does not permanently delete the file from the system.

4.2
Opening Luminex® Results

You can use the menu bar, toolbar, or the drag-and-drop method to open Luminex results (.csv).

Opening Results Using the Menu Bar or Toolbar

1. Click the **Open CSV File** button . Alternatively, select **File → Open CSV File** from the menu bar.
   ⇒ The Open dialog box appears (Figure 4.7).

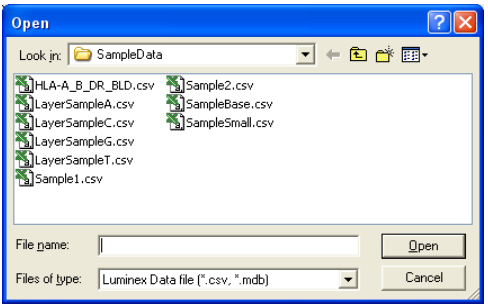


Figure 4.7 Open dialog box

2. Double-click the .csv file that you want to open.
   ⇒ The Project Manager and Project Window appear (Figure 4.8).

   In the Project Manager, the file tree displays the file name. The Project Window displays the Typing table (default).

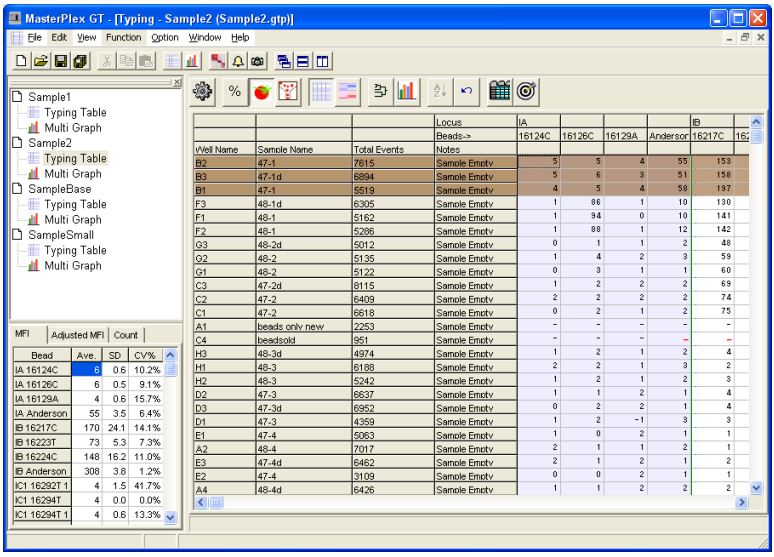


Figure 4.8 Project Manager and Project Window

NOTE: See *The Project Manager and Project Window* on page 4.1 for more information.

Opening Results Using the Drag-and-Drop Method

1. Open Windows Explorer and adjust the window size so that you can view both the MasterPlex™ GT and Windows® Explorer application windows.
2. In Windows Explorer, navigate to the .csv file that you want to open.
3. Select the .csv file, then click and hold the mouse button while you drag the selected file to the MasterPlex GT application window (Figure 4.9).
4. Release the mouse button.

   ⇒ The .csv file opens in MasterPlex GT.

   The Project Manager and Project Window appear (Figure 4.8). In the Project Manager, the file tree displays the file name. The Project Window displays the Typing table (default).

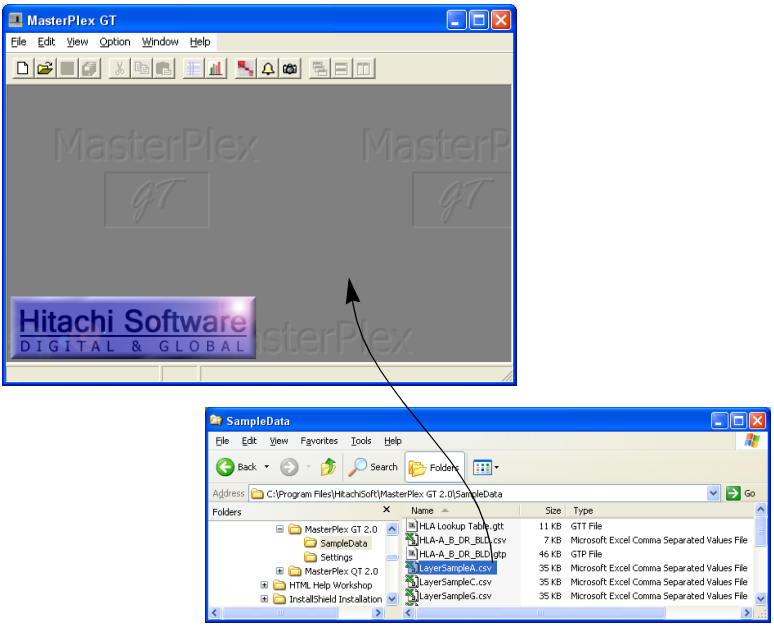


Figure 4.9 MasterPlex GT application window and Windows® Explorer
To open a Luminex® results file, drag the file of interest onto the MasterPlex™ GT application window.

4.3
Merging Results

You can combine or *merge* results and view the merged data in one Typing table. There are two ways to merge results:

- Sample merge - Merges the results from different samples that are probed by identically named bead sets. (See page 3.1 for more information about MasterPlex™ GT bead name conventions.)

  A sample merge enables you to apply the same controls across experiments, compare control data, and compare results across experiments.

- Layer merge - Merges the results from different bead sets (assays) that probe the same sample.

  If an assay format distributes the same sample across several different wells and probes each well with a different bead set, you can use the layer merge function to view the results from the different bead sets in one Typing table.

Sample Merge

Use the sample merge function to combine the results from different samples that use identically named bead sets. There are three ways to perform a sample merge:

- Drag-and-drop method
- Batch method
- Merge wizard

NOTE: Only results from identically named bead sets can be merged using the drag-and-drop method.

Sample Merge Using the Drag-and-Drop Method

1. In the Project Manager, click and hold the file of interest while you drag it to the file that you want to merge it with (Figure 4.10).

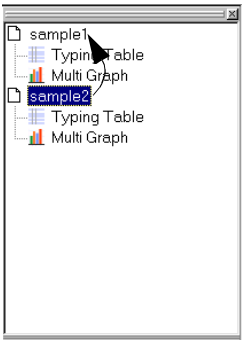


Figure 4.10 Project Manager
Use a drag-and-drop operation to merge results.

2. At the prompt, click **OK**.
   ⇒ The Typing table displays the merged results.

   The sample columns from the dragged file are added to the bottom of the Typing table and the well locations are numbered 2-A1, 2-A2, 2-A3, and so on. If another file is merged, the sample columns are added to the bottom of the table and the well locations are designated 3-A1, 3-A2, 3-A3, and so on.

Sample Merge Using the Batch Method

1. In the Project Manager, right-click the project of interest and select **Merge All** from the shortcut menu that appears (Figure 4.11).
   ⇒ Projects (below the selected project) with bead sets named identical to the bead set of the selected project are merged with the selected project.

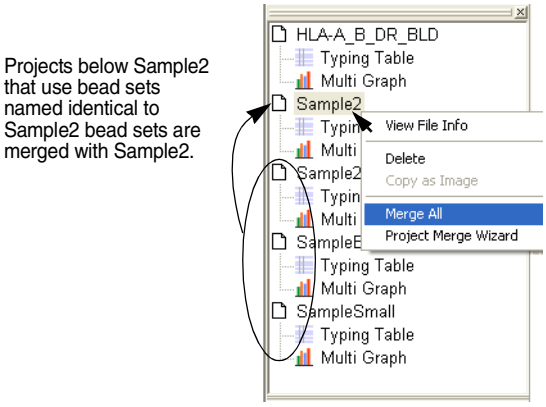


Figure 4.11 Project Manager

Sample Merge Using the Wizard

1. In the Project Manager, right-click the project of interest and select **Project Merge Wizard** from the shortcut menu that appears (Figure 4.12).

   ⇒ The Project Merge Wizard appears (Figure 4.13).

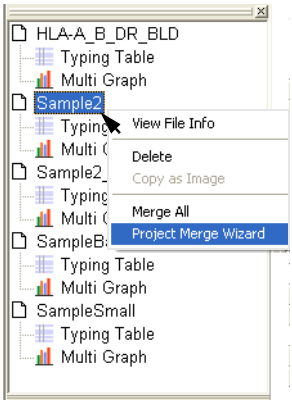


Figure 4.12 Project Manager

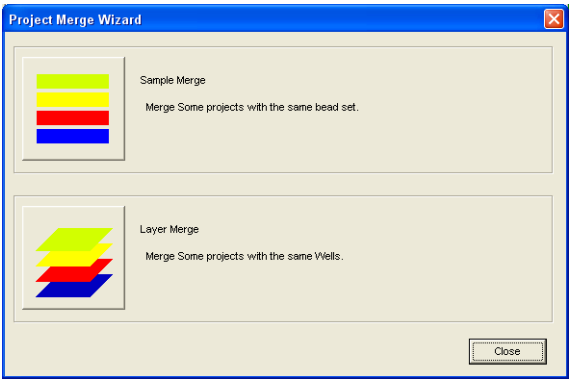


Figure 4.13 Project Merge Wizard

2. Click the **Sample Merge** button .
   ⇒ The wizard displays a drop-down list of projects that are open in the Project Manager (Figure 4.14).

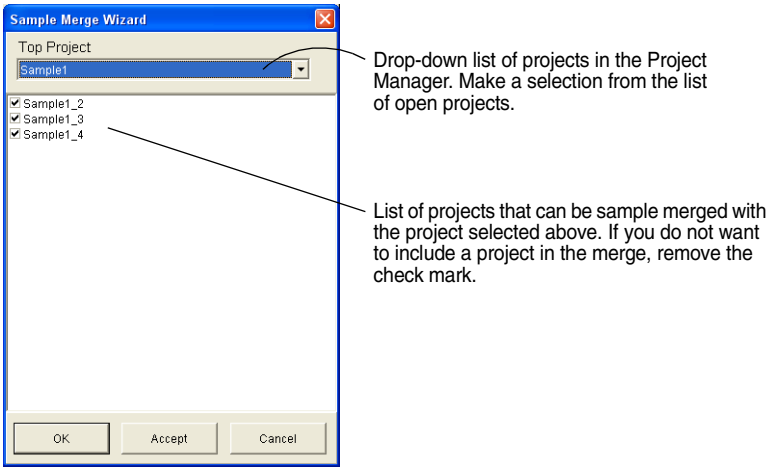


Figure 4.14 Sample Merge Wizard

3. Make a selection from the Top Project drop-down list.
   ⇒ The wizard shows all projects that can be sample merged with the selected project (results that have identically named bead sets). By default, all of the projects are selected for the merge.

4. Confirm the projects selected for the merge. Remove the check mark from a project that you do not want to include in the merge.

   To remove all check marks, right-click the wizard and select **Uncheck All** from the shortcut menu that appears.

   To check mark all of the projects, right-click the wizard and select **Check All** in the shortcut menu.

5. Click **Accept** to merge the projects and keep the Sample Merge wizard open. Click **OK** to merge the projects and close the wizard.

Layer Merge

Use the layer merge function to combine the results from different bead sets that probe the same sample. For example, after a layer merge, the Typing table can display results from more than 100 different bead sets that probe the same sample.

NOTE: To perform a layer merge, a sample must have the same well location across all of the experiments and the bead names must be unique (no two projects can include a bead with the same name).

1. In the Project Manager, right-click the project of interest and select **Project Merge Wizard** from the shortcut menu that appears (Figure 4.15).

   ⇒ The Project Merge Wizard appears (Figure 4.16).

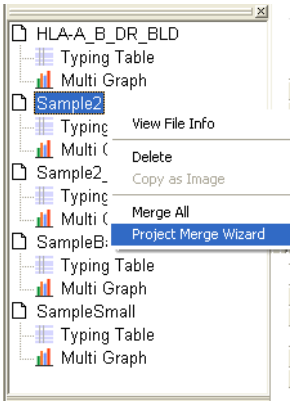


Figure 4.15 Project Manager

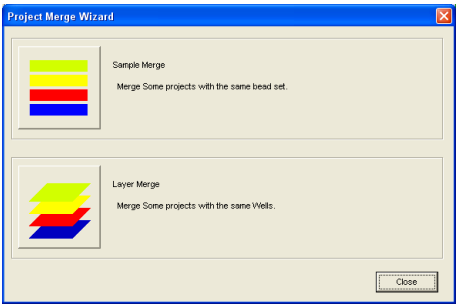


Figure 4.16 Project Merge Wizard

2. Click the **Layer Merge** button .
   ⇒ The wizard displays a drop-down list of results open projects (Figure 4.17).

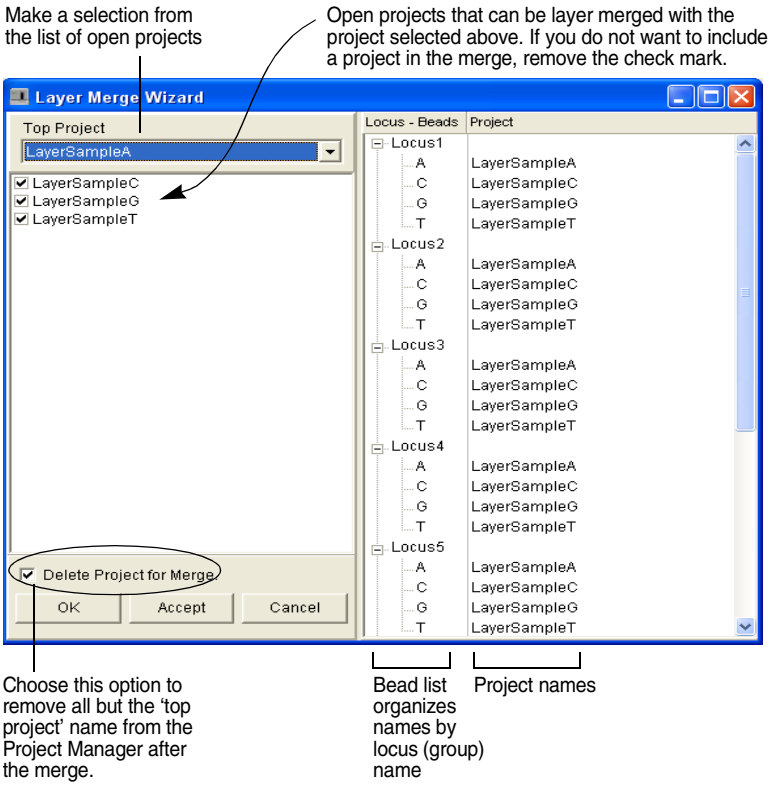


Figure 4.17 Layer Merge Wizard

3. Make a selection from the Top Project drop-down list (Figure 4.17).

   ⇒ The wizard shows all open projects that can be merged with the selected project (each sample has the same well location across all experiments). By default, all of the projects are selected for the merge.

4. Confirm the projects selected for the merge or remove the check mark from a project that you do not want to include in the merge.

   To remove all check marks, right-click the wizard and select **Uncheck All** from the shortcut menu that appears.

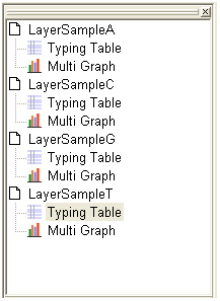
   To check mark all of the projects, right-click the wizard and select **Check All** in the shortcut menu.

NOTE: If a project includes a bead name that is used in another project, the Project Merge wizard displays the name in red. If this occurs, the merge cannot proceed. For information on how to edit a bead name, see *Editing a Bead Name* on page 4.19.

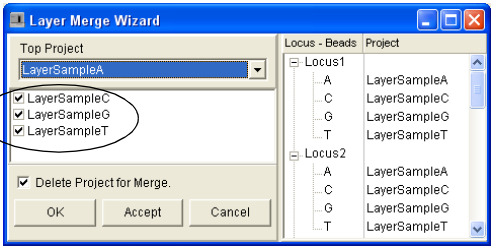
5. Choose the **Delete Project for Merge** option to remove all but the 'top project' name from the Project Manager after the merge.

   For example, in Figure 4.18, only the project LayerSampleA will be displayed in the Project Manager after the merge.

Project Manager shows all open projects.



Projects selected for layer merge with LayerSample A. (top project).



If you choose **Delete Project for Merge**, the Project Manager shows only the top project name after the merge.

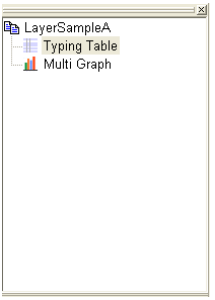


Figure 4.18 Project Manager

6. Click **Accept** to merge the projects and keep the Layer Merge wizard open. Click **OK** to merge the projects and close the wizard.

Editing a Bead Name

You can edit a bead name in the bead list of the Layer Merge wizard. Bead name components include: Locus (group) + Allele + Suffix. (The default is no suffix.)

1. In the Layer Merge wizard, right-click the bead name that you want to edit and select **Edit Bead Name** from the shortcut menu that appears (Figure 4.19).

   ⇒ The edit bead name dialog box appears (Figure 4.20).

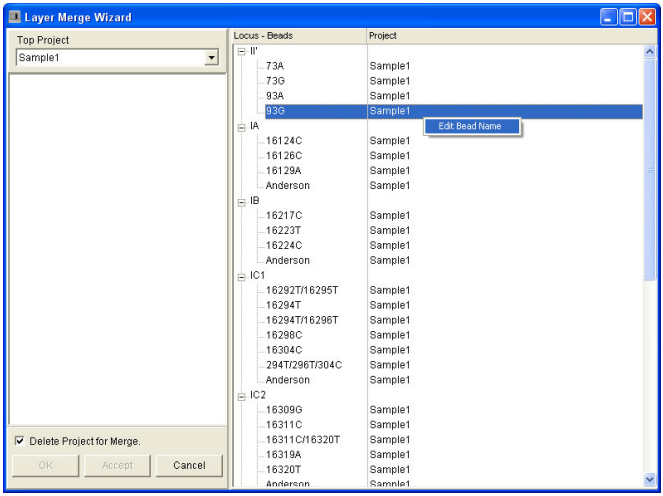


Figure 4.19 Layer Merge wizard

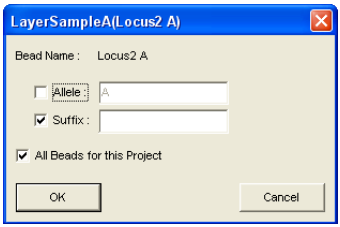


Figure 4.20 Edit bead name dialog box

The box title is the selected project (LayerSampleA) and bead name (Locus2 A).

2. To edit the allele name, choose the **Allele** option and enter an allele name.
3. To edit the suffix name, choose the **Suffix** option and enter a suffix name.
4. To apply the new allele and suffix names to all loci in the project, choose the **All Beads for this Project** option.
5. Click **OK**.

CHAPTER 5

NEGATIVE CONTROLS

This chapter explains negative controls and how to set them. A negative control (NC) can be set manually in the Typing table or the Multi Graph view. The MasterPlex™ GT software can also automatically identify negative controls based on keyword recognition.

5.1 Local and Global Negative Controls

The MasterPlex GT software computes a background value (the average MFI of the negative controls) and subtracts it from the sample (bead set) MFI to obtain the background-adjusted sample MFI data.

A *local* negative control is specific to a particular set of result. A *global* negative control is applied to merged results. Both local and global negative controls can be applied to a results file.

The MasterPlex GT software first determines if global negative controls have been specified and computes:

$$\text{Global NC} = (\text{Global NC}_1 + \text{Global NC}_2 + \ldots \text{Global NC}_n)/n$$

Next, the software examines each results file for local negative controls (local NC). The background value for a results file is the average of the local NCs and global NC.

Table 5.1 on page 5.2 shows example results files and negative controls, and how the MasterPlex GT software computes the background value for each results file. In this example:

$$\text{Global NC} = (\text{Global NC}_a + \text{Global NC}_b + \text{Global NC}_c)/3$$

The MasterPlex GT software subtracts the computed background value from the sample (bead set) MFI to obtain the background-adjusted sample MFI data.

Table 5.1 Computed background values

Results	Local Negative Controls	Global Negative Controls	Computed Background Value
Plate1	Local NC_a, Local NC_b		(Local NC_a + Local NC_b + Global NC[a])/3
Plate2	Local NC_c		(Local NC_c + Global NC)/2
Plate3	None		Global NC
Plate4		Global NC_a	Global NC
Plate5		Global NC_b, Global NC_c	Global NC

a. Global NC = (Global NC_a + Global NC_b + Global NC_c)/3

5.2
Setting Negative Controls Manually

If you are working with one set of results, you can set a *local* negative control. If you are working with merged results, you can set a *global* negative control that is applied to all of the merged data.

1. To create negative controls, in the Typing table, click the names of the samples that you want to designate negative controls. There are three ways to do this:
   - Click a sample name.
   - To select adjacent sample names, click and hold the mouse while you move the pointer over the sample names. Click the mouse when the complete selection is highlighted. Alternatively, press and hold the **Shift** key while you click the first and last sample name in the selection.
   - To select nonadjacent samples, press and hold the **Ctrl** key while you click the sample names.

   ⇒ The selected sample rows are highlighted.

2. To set the selected samples as negative controls, right-click a highlighted sample name and do either of the following:
   - If you are working with one results file, click **Local Negative Control** in the shortcut menu that appears.
   - If you are working with merged results, click **Global Negative Control** in the shortcut menu that appears.

   ⇒ The selected samples (rows) are designated negative controls and the rows display dash marks (-) (Figure 5.1).

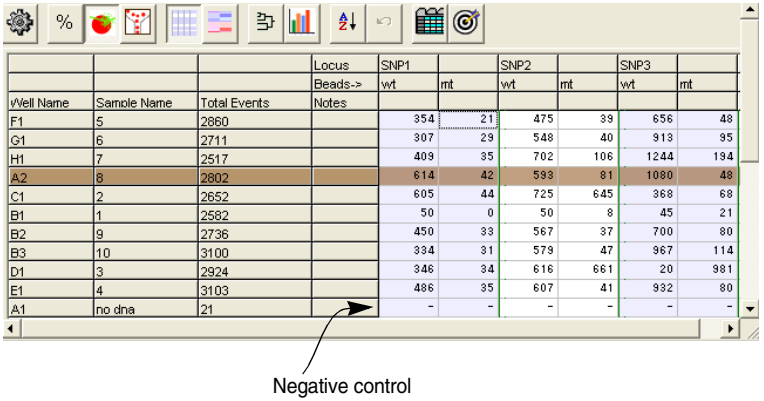


Figure 5.1 Typing table
The sample named no dna (well A1) is a negative control.

Removing a Negative Control

1. Select the negative control sample row(s).

   ⇒ The selected sample row(s) are highlighted.
2. Right-click a sample name in the highlighted selection and do either of the following:
   - If you are working with one results file, click **Local Negative Control** in the shortcut menu that appears.
   - If you are working with merged files, click **Global Negative Control** in the shortcut menu that appears.

   ⇒ The dash marks (-) are replaced with the results data.

5.3
Setting Negative Controls Automatically

The MasterPlex™ GT software can automatically set the negative controls by identifying user-specified key words in the sample name. In the

Background tab of the Application dialog box, you can set the keywords that identify a negative control.

1. Select **Option → Set Application Options** from the menu bar.
   ⇒ The Application Options dialog box opens (Figure 5.2).

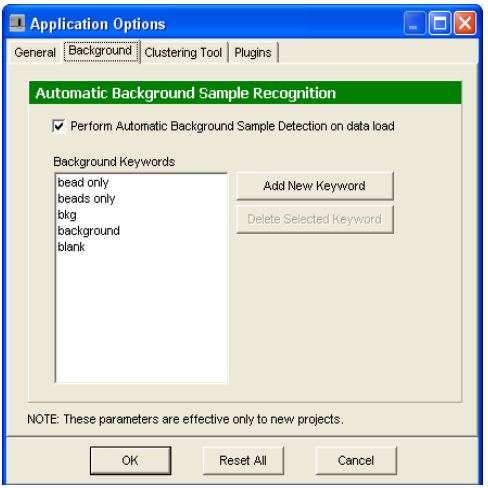


Figure 5.2 Application Options dialog box, Background tab

2. Click the Background tab.
3. Choose the option **Perform Automatic Background Sample Detection on data load**.
4. To define a keyword, click **Add New Keyword,** enter the keyword in the dialog box that appears, and click **OK** (Figure 5.3).
   ⇒ The keyword is added to the Background Keywords list (Figure 5.2).

NOTE: A keyword added during a session is applied only to subsequently opened results.

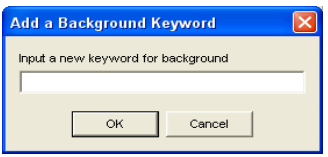


Figure 5.3 Add a Background Keyword dialog box

5. To delete a keyword, select the keyword you want to delete in the Background Keywords list and click **Delete Selected Keyword**. At the prompt, click **Yes**.

The MasterPlex™ GT software can use median fluorescence intensity (MFI) or relative intensity (RI) data to call alleles. This chapter explains how to set parameters for the allele calling algorithm.

6.1
Parameter Settings and Options

- To display the parameter settings, click the **Parameter Setting** button .

  ⇒ The Parameter Setting dialog box appears (Figure 6.1).

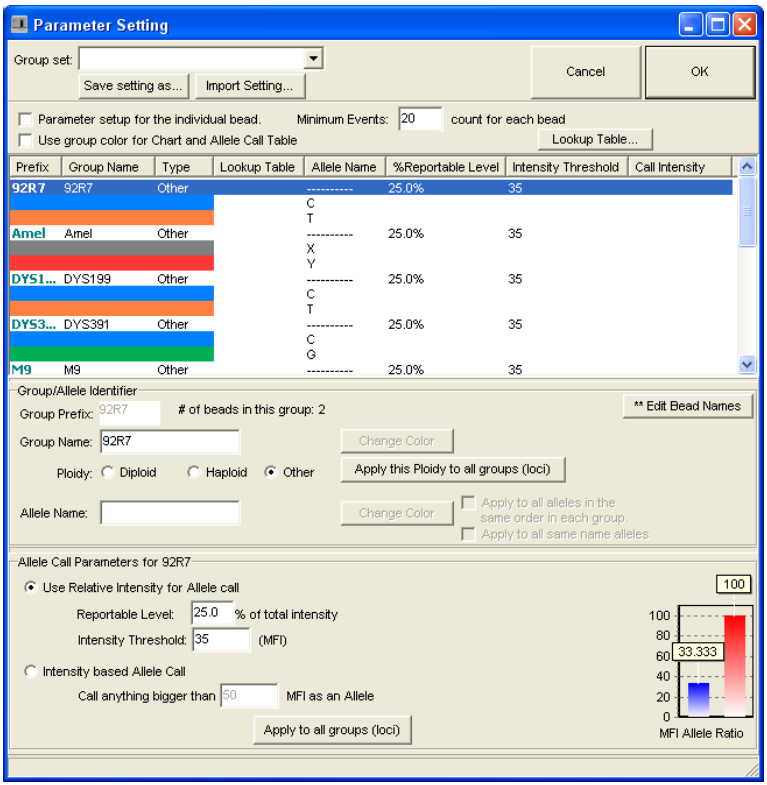


Figure 6.1 Parameter Setting dialog box

Parameter settings and options include:

Parameter setup for the individual bead	Choose this option to apply the parameter settings to a user-selected allele (bead type). If this option is not chosen, the group parameters are applied to alleles on a per group basis.
Use group color for Chart and Allele Call Table	Choose this option to apply the group color to the allele data in the Allele Call table, Multi Compare graph, and Depth graph (alleles of the same locus (group) are represented by the same color). If this option is not chosen, each allele is represented by a different color.
Minimum Events	The minimum number of beads that should be counted in the Luminex® system for each bead type in each sample.
Ploidy	The ploidy may be specified for all groups (loci) or for individual groups. The ploidy affects the allele frequency calculation. (Note: Other and Haploid ploidy are the same.)
Use Relative Intensity (RI) for Allele Call (see See "Relative Intensity Allele Call" on page 6.3)	The software calls the allele if all of the following conditions are met: • $RI_{allele} \geq$ user-specified RI threshold • $MFI_{allele} \geq$ user-specified intensity threshold
Intensity Based Allele Call (see See "Intensity-Based Allele Call" on page 6.5)	The software calls the allele if both of the MFI_{allele} > user-specified absolute intensity threshold

NOTE: If the bead count is less than the number of minimum events specified in the Parameter Settings dialog box, the Typing table displays the bead count data in red.

6.2 Ploidy

You can specify diploid, haploid, or other ploidy for each group (locus). The ploidy affects the allele frequency calculation (see *Allele Frequency* on page 7.20). If you select diploid and there is only one allele called, the allele will be shown twice in the Allele Call table.

NOTE: The Haploid option and Other option are the same.

6.3 Relative Intensity Allele Call

For this method, the MasterPlex™ GT software computes the relative intensity (RI) of each allele in a group. For example, in a biallelic analysis of allele a and allele b:

$$RI_a = [MFI_a / (MFI_a + MFI_b)] \times 100$$
$$RI_b = [MFI_b / (MFI_a + MFI_b)] \times 100$$

The software calls an allele if the following conditions are met:

- $RI_{allele} \geq$ a user-specified RI threshold (*reportable level*)
- $MFI_{allele} \geq$ a user-specified intensity threshold

1. Click the button to display the Parameter Setting dialog box (Figure 6.2).

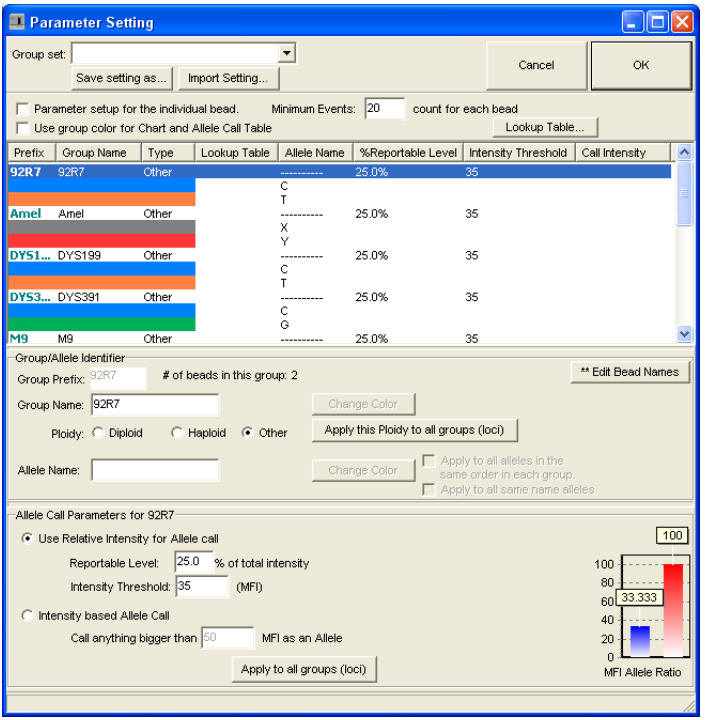


Figure 6.2 Parameters Setting dialog box

2. If you want to set parameters for one particular allele (bead type) only, select the allele name in the upper box, and choose the option **Parameter setup for the individual bead**.

NOTE: If this option is not chosen, the parameter settings are applied to alleles on a per group basis.

3. Confirm the default or enter a new **Minimum Events** value.
4. Choose the **Use Relative Intensity for Allele Call** option.
5. Confirm the default or enter a new Reportable Level.

   When the analysis is biallelic, the Parameter Setting dialog box also displays a bar graph of the MFI allele ratio specified by the reportable level. For example, the parameter settings in (Figure 6.3) call an allele if $MFI_a / (MFI_a + MFI_b) \times 100 \geq 25\%$.

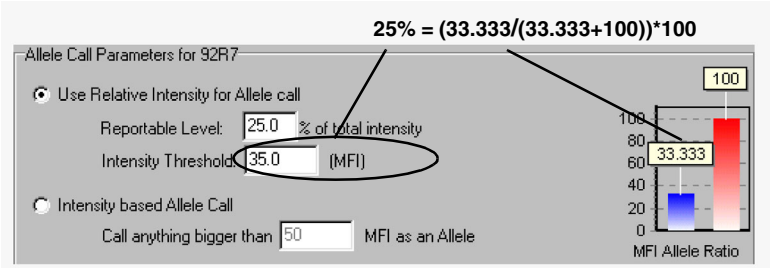


Figure 6.3 MFI allele ratio
MFI allele ratio shows the minimum biallele ratio required to meet the reportable level.

6. Confirm the default or enter a new Intensity Threshold (MFI).
7. Click **Apply**.
   ⇒ The parameter settings are applied to the active results.

NOTE: You can also set the relative intensity thresholds in the Thresholds tab of the Multi Graph view. For more information, see *Threshold Editing* on page 8.16.

6.4
Intensity-Based Allele Call

For this method, the MasterPlex™ GT software calls an allele if the MFI is greater than a user-specified value.

1. Click the button to display the Parameter Setting dialog box (Figure 6.2).
2. If you want to set parameters for one particular allele (bead type) only, select the allele name in the upper box, and choose the **Parameter setup for the individual bead** option.

NOTE: If this option is not chosen, the parameter settings are applied to alleles on a per group basis.

3. Confirm the default or enter a new **Minimum Events** value.
4. Choose the **Intensity Based Allele Call** option.
5. Confirm the default or enter a new MFI threshold value.
6. Click **Apply**.
   ⇒ The parameter settings are applied to the active results.

6.5
Editing a Bead Name

You can edit the bead name. (For more information about prefix, group and allele names, see *Bead Name Conventions* on page 3.1.)

1. Click the button to display the Parameter Setting dialog box ((Figure 6.5)).
2. Click **Edit Bead Names**.
   ⇒ The Edit Bead Names dialog box opens (Figure 6.4).

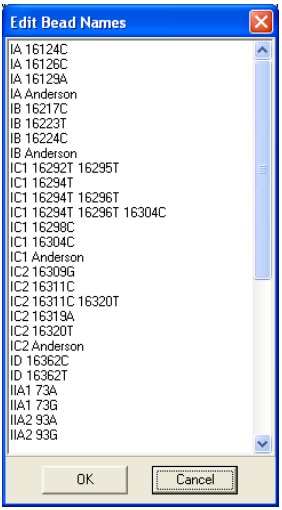


Figure 6.4 Edit Bead Names dialog box

3. Select the bead name you want to edit and enter a new name.
4. Click **OK** when you finish editing the names.
   ⇒ The new bead name (prefix, group, and allele name) is displayed in the Parameter Setting dialog box, Typing table, graph view, and statistics table.

Editing the Group or Allele Name Only

You can also edit just the group or allele name in the Parameter Setting dialog box.

To edit the group name:

1. Click the button to display the Parameter Setting dialog box ((Figure 6.5)).
2. Select the group name you want to edit (Figure 6.5).
3. Enter the new group name in the Group Name box and click **Apply**.

To edit the allele name:

1. Select the allele name you want to edit (Figure 6.6).
2. Enter the new allele name in the Allele Name box (Figure 6.6) and click **Apply**.

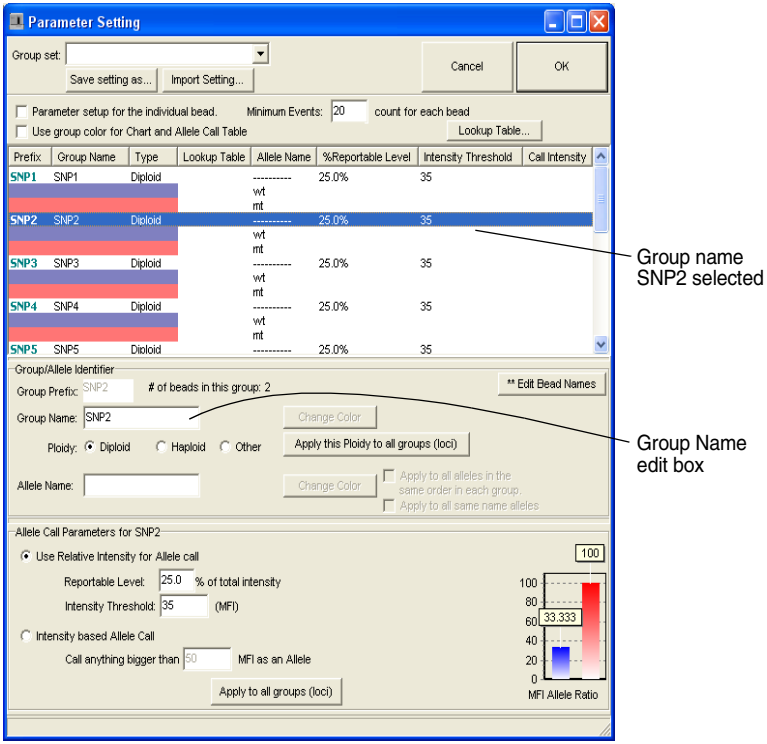


Figure 6.5 Parameter Setting dialog box
Group name SNP2 selected.

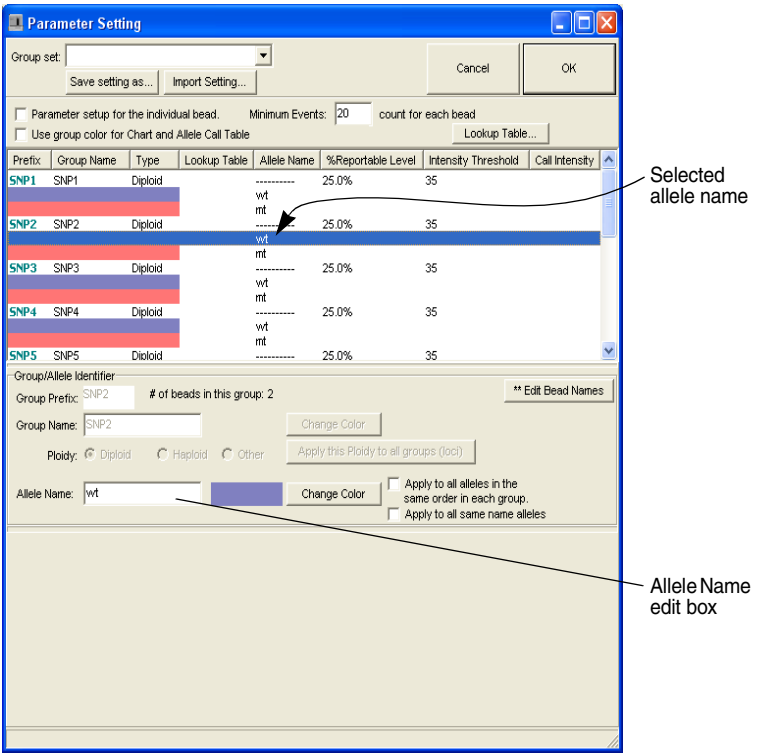


Figure 6.6 Parameter Setting dialog box
Allele name wt selected.

6.6
Group and Allele Color

If you choose the option **Use group color for Chart and Allele Call Table** in the Parameter Setting dialog box (Figure 6.6), the MasterPlex™ GT software assigns one *group* color to all of the alleles in a group (locus). As a result, the:

- Multi Compare bar graph displays the allele data using the group color
- Depth bar graph displays the allele data using the group color
- Allele Call table displays the group colors

If this option is not chosen, the software assigns a different color to each allele. You can change the default group or allele color.

Changing the Group or Allele Color

1. Click the button to display the Parameter Setting dialog box (Figure 6.7).

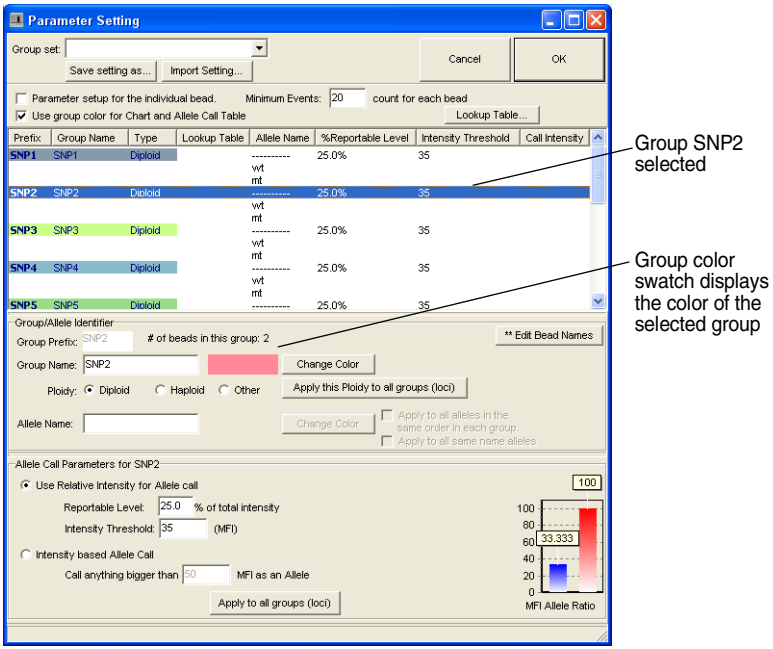


Figure 6.7 Parameter Setting dialog box
Group SNP2 selected.

2. To change a group color, choose the option **Use group color for Chart and Allele Call Table**. To change an allele color, do not choose this option.
3. Click the group name or allele name with the color that you want to change.
   ⇒ The Group Name color swatch (or Allele Name color swatch) shows the selected color (Figure 6.7 and Figure 6.8).

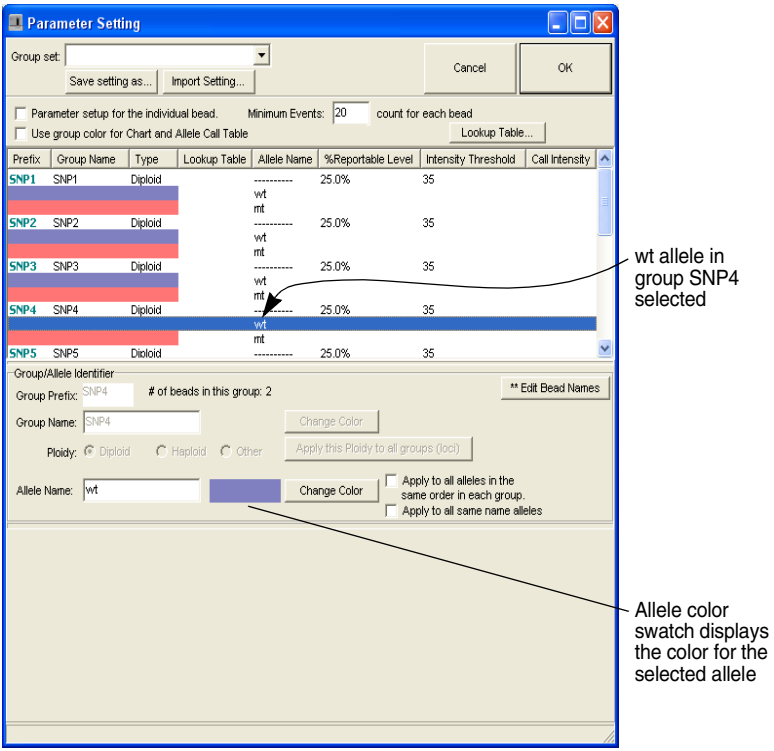


Figure 6.8 Parameter Setting dialog box
wt allele in group SNP4 selected.

4. Two options are available when setting allele color:
   - If you want to apply the color to the same position number allele in all groups (for example, the first allele in each group), choose the option **Apply to alleles in the same order in each group**.
   - If you want to apply the color to all alleles with the same name (in all groups), choose the **Apply to same name alleles** option.
5. Click **Change Color**.

   ⇒ The color palette appears (Figure 6.9).

Figure 6.9 Color palette

6. To select a predefined color, click one of the basic colors.
7. To define a custom color, click **Define Custom Colors**.
   ⇒ The color palette shows the custom color options (Figure 6.10).

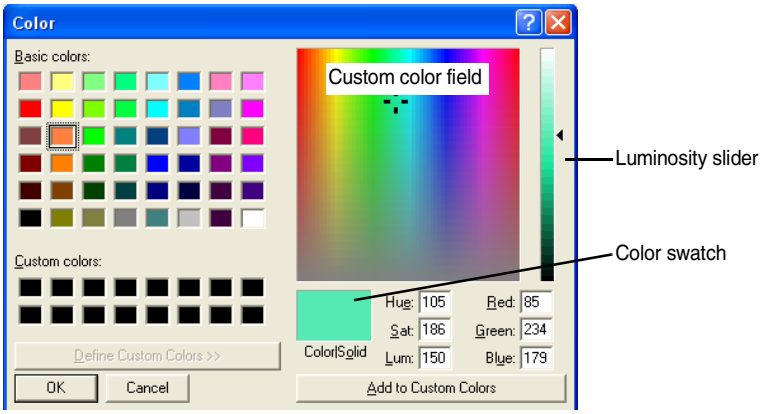


Figure 6.10 Color palette
Custom color options.

8. To define a color, use the click-and-drag operation to move the cross hairs in the custom color field. Adjust the color brightness using the luminosity slider.
   ⇒ The Color swatch shows the color selection.

9. When you are finished defining the color, click **Add to Custom Colors** to apply the color, and click **OK**.
   ⇒ If you selected a group name, the new group color is displayed in the Group Name color swatch and is applied to the data for the alleles of this group in the Multi Compare, Depth graph, and Allele Call table.

   If you selected an allele name, the new allele color is displayed in the Allele Name color swatch and is applied to the data for the selected allele in the Multi Compare, Depth graph, and Allele Call table.

6.7
Working With Group Sets

You can save the settings in the Parameter Setting dialog box (Figure 6.12) as a *group set* (.xml) for use in future sessions. You may also import a previously saved group set.

Creating a Group Set

1. Open the Parameter Setting dialog box (click the button).
2. Click **Save setting as**.
   ⇒ The Create a New Group Set dialog box appears (Figure 6.11).

Figure 6.11 Create a New Group Set dialog box

3. Enter a name for the group set and click **OK**.
   ⇒ The group set (.xml) is saved and the name is added to the New Group Set name drop-down list (Figure 6.12).

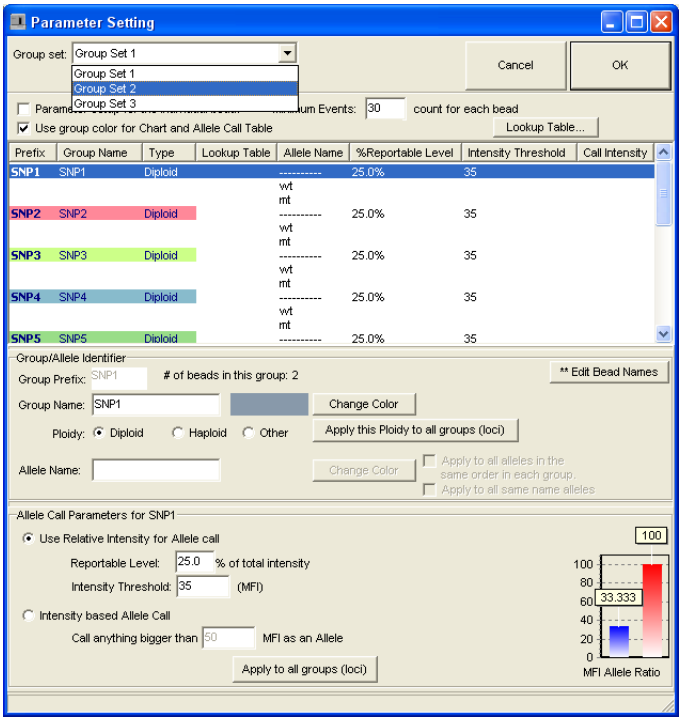


Figure 6.12 Parameter Setting dialog box
Group set drop-down list displays available group sets.

Selecting a Group Set

To select a group set for the active results, click the Group set box, and make a selection from the drop-down list.

Importing a Group Set

You can import a previously saved group set (for example, a group set created on another system).

1. Click **Import Setting.**
   ⇒ The Open dialog box appears (Figure 6.13).

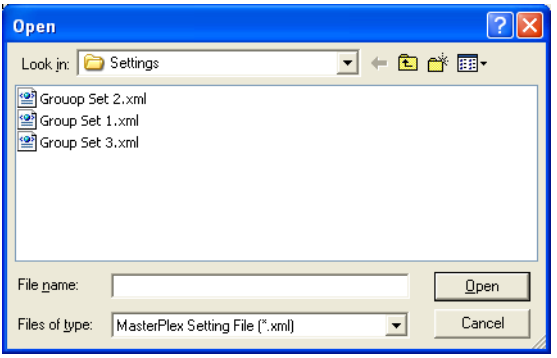


Figure 6.13 Open dialog box
Group set files (.xml).

2. Double-click the group set file (.xml) for import.
   ⇒ The Parameter Setting dialog box displays the imported group set.

6.8
Saving a Project

The contents of the Project Window can be saved as a project (.gtp). A project includes the:

- results file(s) (.csv) in the Typing table
- graphs or dendrogram created in the Multi Graph view
- parameter settings
- user-selected samples

1. Click the **Save** button . Alternatively, select **File → Save Project** from the menu bar.
   ⇒ The Save As dialog box appears (Figure 6.14).

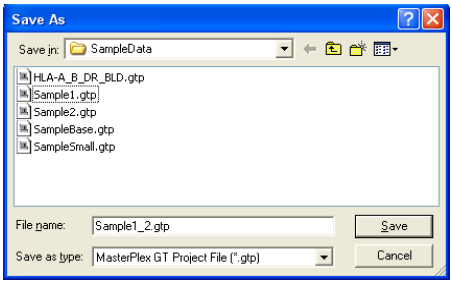


Figure 6.14 Save As dialog box

2. Confirm the default or enter a new location where you want to save the file.
3. Enter a file name for the project (.gtp) and click **Save**.

Using the Save As Function

Use the Save As function if you change the project (for example, change a parameter setting or option) and want to save it without overwriting the previously saved file (.gtp).

1. Select **File → Save Project As** from the menu bar.
   ⇒ The Save As dialog box appear (Figure 6.14).
2. Confirm the default or enter a new location where you want to save the project.
3. Enter a file name for the project (.gtp) and click **Save**.

Opening a Project

You can use the menu bar, toolbar, or a drag-and-drop method to open a project (.gtp).

Using the Menu Bar or Toolbar

1. To open a previously saved project (.gtp), click the **Open Project File** button. Alternatively, select **File → Open Project File** from the menu bar.
   ⇒ The Open dialog box appears (Figure 6.15).

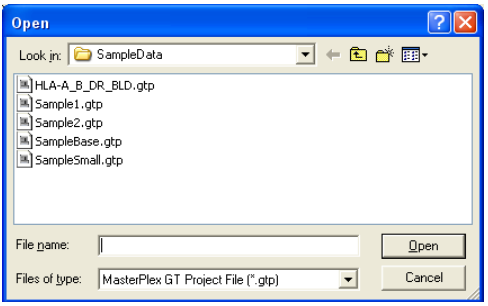


Figure 6.15 Open dialog box
Project files(.gtp).

2. Double-click the project file (.gtp) you want to open.
   ⇒ The project window for the selected file opens.

Using the Drag-and-Drop Method

1. Navigate to the project file (.gtp) you want to open.
2. Use the drag-and-drop operation to place the file in the MasterPlex™ GT application window (Figure 6.16).
   ⇒ The Project Manager and Project Window appear (Figure 6.16).

   In the Project Manager, the file tree displays the file name. The Project Window displays the Typing table (default).

NOTE: For more information about the Project Manager and Project window, see page 4.1.

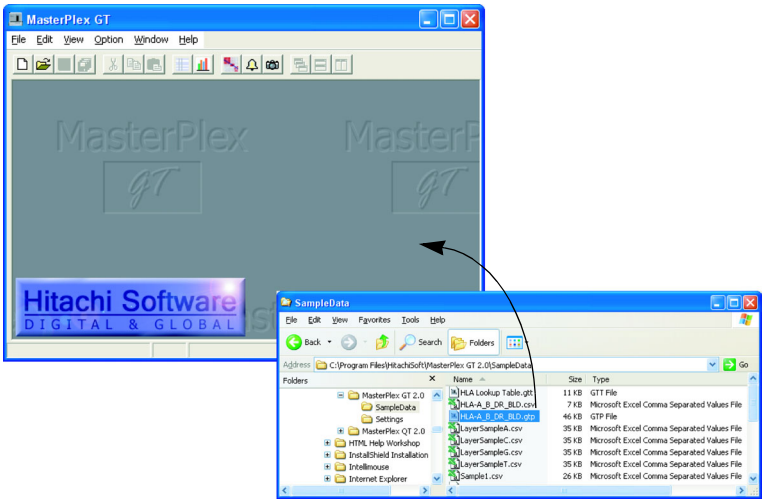


Figure 6.16 MasterPlex GT window and Windows Explorer
To open a project (.gtp), drag the file onto the MasterPlex GT application window.

CHAPTER 7

RESULTS TABLES

The MasterPlex™ GT software shows results in several tabular formats, including the Typing table, Allele Call table, and Homology table. This chapter explains how to view the tables and the data available in each.

7.1 The Typing Table

In the Typing table, you can:

- view median fluorescence intensity (MFI), relative intensity (RI), or bead count data
- quickly assess assay performance
- designate negative controls
- compare results across experiments
- sort samples by expression level or genotype

This section explains the different data views in the Typing table and its functional features.

Viewing the Typing Table

The Typing table is the default view when you open a results file or project. To display the Typing table for:

- the active project, click the  button
- a particular project, click **Typing Table** under the project of interest in the in the Project Manager (Figure 7.1)

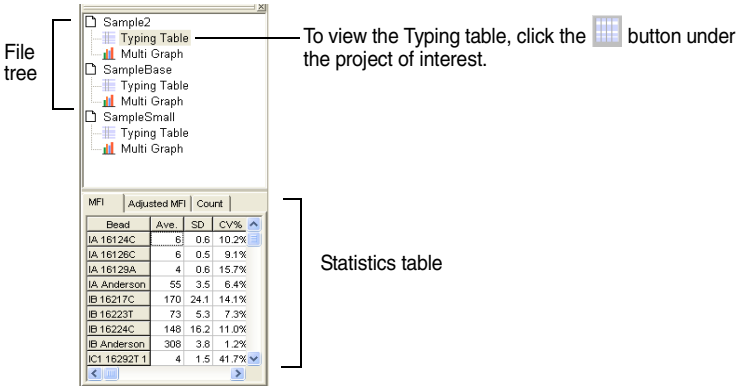
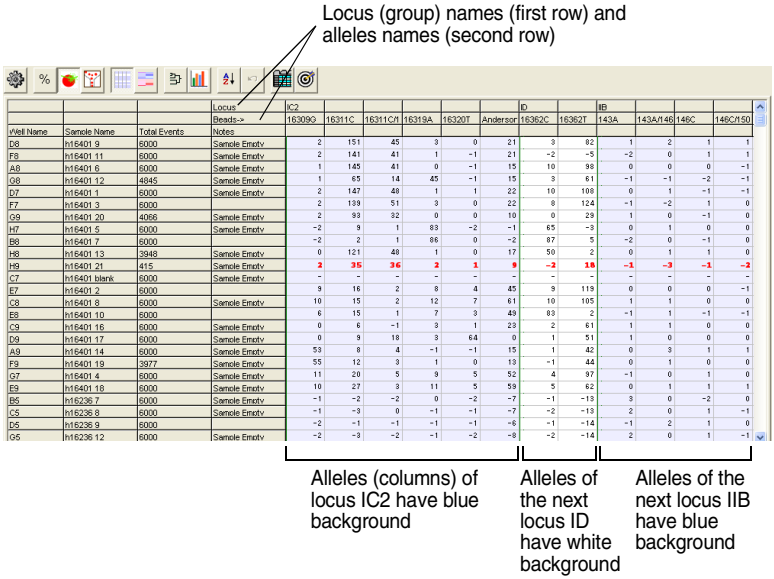


Figure 7.1 Project Manager

The Typing table displays the MFI and bead count data for the open results. If the bead set names follow the MasterPlex™ GT naming convention, the Typing table automatically organizes the data by loci and alleles (Figure 7.2). (See page 3.1 for more information about the bead naming convention.)

If you merge results, the Typing table displays all of the results. (See page 4.11 for more information on merging results.)



			Locus	IC2						ID		IIB			
			Beads->	16309Q	16311C	16311C/T	16319A	16320T	Andersor	16362C	16362T	143A	143A/146	146C	146C/150
Well Name	Sample Name	Total Events	Notes												
D8	h16401 9	6000	Sample Empty	2	151	45	3	0	21	3	82	1	2	1	1
F8	h16401 11	6000	Sample Empty	2	141	41	1	-1	21	-2	-5	-2	0	1	1
A8	h16401 6	6000	Sample Empty	1	145	41	0	-1	15	10	98	0	0	0	-1
G6	h16401 12	4845	Sample Empty	1	65	14	45	-1	15	3	61	-1	-1	-2	-1
D7	h16401 1	6000	Sample Empty	2	147	48	1	1	22	10	108	0	1	-1	-1
F7	h16401 3	6000		2	139	51	3	0	22	8	124	-1	-2	1	0
G9	h16401 20	4066	Sample Empty	2	93	32	0	0	10	0	29	1	0	-1	0
H7	h16401 5	6000	Sample Empty	-2	9	1	83	-2	-1	65	-3	0	1	0	0
B8	h16401 7	6000		-2	2	1	86	0	-2	87	5	-2	0	-1	0
H8	h16401 13	3948	Sample Empty	0	121	40	1	0	17	50	2	0	1	1	0
H9	h16401 21	415	Sample Empty	**3**	**35**	**36**	**2**	**1**	**9**	**-2**	**18**	**-1**	**-3**	**-1**	**-2**
C7	h16401 blank	6000	Sample Empty	-	-	-	-	-	-	-	-	-	-	-	-
E7	h16401 2	6000		9	16	2	8	4	45	9	119	0	0	0	-1
C8	h16401 8	6000	Sample Empty	10	15	2	12	7	61	10	105	1	1	0	0
E8	h16401 10	6000		6	15	1	7	3	49	83	2	-1	1	-1	-1
C9	h16401 16	6000	Sample Empty	0	6	-1	3	1	23	2	61	1	1	0	0
D9	h16401 17	6000	Sample Empty	0	9	18	3	64	0	1	51	1	0	0	0
A9	h16401 14	6000	Sample Empty	53	8	4	-1	-1	15	1	42	0	3	1	1
F9	h16401 19	3977	Sample Empty	55	12	3	1	0	19	-1	44	0	1	0	0
G7	h16401 4	6000	Sample Empty	11	20	5	9	5	52	4	97	-1	0	1	0
E9	h16401 18	6000	Sample Empty	10	27	3	11	5	59	5	62	0	1	1	1
B5	h16236 7	6000	Sample Empty	-1	-2	-2	0	-2	-7	-1	-13	3	0	-2	0
C5	h16236 8	6000	Sample Empty	-1	-3	0	-1	-1	-7	-2	-13	2	0	1	-1
D5	h16236 9	6000		-2	-1	-1	-1	-1	-6	-1	-14	-1	2	1	0
G5	h16236 12	6000	Sample Empty	-2	-3	-2	-1	-2	-8	-2	-14	2	0	1	-1

Figure 7.2 Typing table
Stripe background view uses alternating color (blue or white) to identify the alleles (columns) associated with a locus.

You can select different data views in the Typing table:

Relative intensity (Figure 7.4, page 7.5)	Displays the percent relative intensity for the alleles at a locus.
Median fluorescence intensity (MFI) (Figure 7.5, page 7.6)	Displays the background-adjusted MFI of the alleles.
Bead count (Figure 7.6, page 7.7)	Displays the number of bead events counted for sample acquisition.

You can view the Typing table with a striped (Figure 7.2) or a gradient background (Figure 7.3).

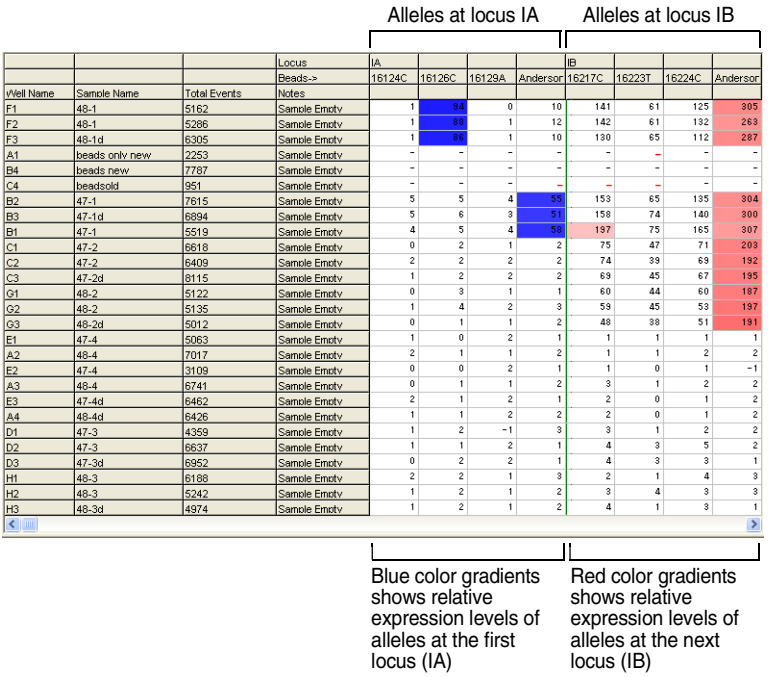
- To view the Typing table with the striped background, click the button.

  ⇒ The alleles (columns) associated with a locus appear blue or white in the Typing table (Figure 7.2).

- To view the Typing table with the gradient background, click the button.

  ⇒ The Typing table highlights the allele relative intensity using a color gradient ((Figure 7.3))

  A darker color shade indicates a higher relative intensity. Red and blue distinguish between the loci.



			Locus	IA				IB			
			Beads->	16124C	16126C	16129A	Andersor	16217C	16223T	16224C	Andersor
Well Name	Sample Name	Total Events	Notes								
F1	48-1	5162	Sample Empty	1	94	0	10	141	61	125	305
F2	48-1	5286	Sample Empty	1	88	1	12	142	61	132	263
F3	48-1d	6305	Sample Empty	1	86	1	10	130	65	112	287
A1	beads only new	2253	Sample Empty	-	-	-	-	-	-	-	-
B4	beads new	7787	Sample Empty	-	-	-	-	-	-	-	-
C4	beadsold	951	Sample Empty	-	-	-	-	-	-	-	-
B2	47-1	7615	Sample Empty	5	5	4	55	153	65	135	304
B3	47-1d	6894	Sample Empty	5	6	3	51	158	74	140	300
B1	47-1	5519	Sample Empty	4	5	4	58	197	75	165	307
C1	47-2	6618	Sample Empty	0	2	1	2	75	47	71	203
C2	47-2	6409	Sample Empty	2	2	2	2	74	39	69	192
C3	47-2d	8115	Sample Empty	1	2	2	2	69	45	67	195
G1	48-2	5122	Sample Empty	0	3	1	1	60	44	60	187
G2	48-2	5135	Sample Empty	1	4	2	3	59	45	53	197
G3	48-2d	5012	Sample Empty	0	1	1	2	48	38	51	191
E1	47-4	5063	Sample Empty	1	0	2	1	1	1	1	1
A2	48-4	7017	Sample Empty	2	1	1	2	1	1	2	2
E2	47-4	3109	Sample Empty	0	0	2	1	1	0	1	-1
A3	48-4	6741	Sample Empty	0	1	1	2	3	1	2	2
E3	47-4d	6462	Sample Empty	2	1	2	1	2	0	1	2
A4	48-4d	6426	Sample Empty	1	1	2	2	2	0	1	2
D1	47-3	4359	Sample Empty	1	2	-1	3	3	1	2	2
D2	47-3	6637	Sample Empty	1	1	2	1	4	3	5	2
D3	47-3d	6952	Sample Empty	0	2	2	1	4	3	3	1
H1	48-3	6188	Sample Empty	2	2	1	3	2	1	4	3
H2	48-3	5242	Sample Empty	1	2	1	2	3	4	3	3
H3	48-3d	4974	Sample Empty	1	2	1	2	4	1	3	1

Figure 7.3 Typing table
Gradient background indicates relative expression levels of the alleles at a locus.

Relative Intensity View

In this view, the Typing table displays the percent of the total intensity (RI) for each allele at a particular locus. In a biallelic analysis, the relative intensity of allele a and allele b is computed as follows:

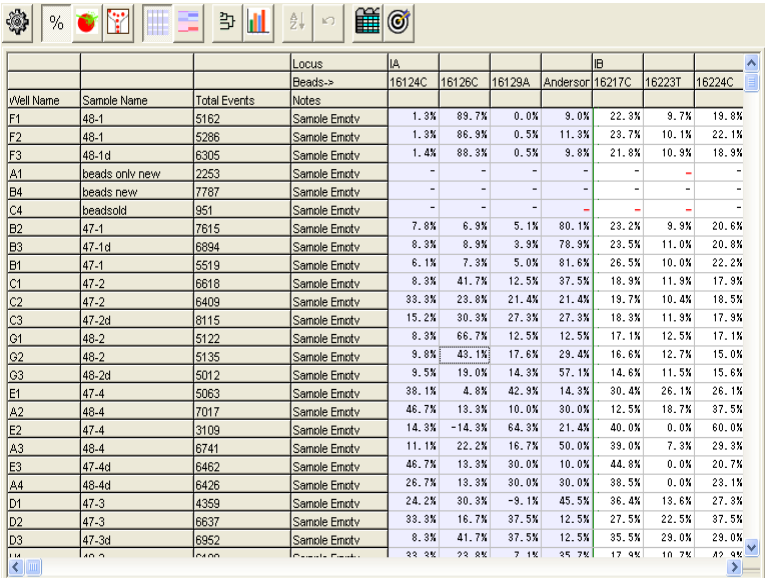
$$RI_a = MFI_a/(MFI_a + MFI_b) \times 100$$

$$RI_b = MFI_b/(MFI_a + MFI_b) \times 100$$

- To display the relative intensity view, click the % button.

  ⇒ The Typing table displays percent relative intensity for each allele represented in the bead set (Figure 7.4).

NOTE: RI data displayed in red indicates the bead count was less than the user-specified event number set in the Parameter Setting dialog box.



			Locus	IA				IB		
			Beads->	16124C	16126C	16129A	Anderson	16217C	16223T	16224C
Well Name	Sample Name	Total Events	Notes							
F1	48-1	5162	Sample Empty	1.3%	89.7%	0.0%	9.0%	22.3%	9.7%	19.8%
F2	48-1	5286	Sample Empty	1.3%	86.9%	0.5%	11.3%	23.7%	10.1%	22.1%
F3	48-1d	6305	Sample Empty	1.4%	88.3%	0.5%	9.8%	21.8%	10.9%	18.9%
A1	beads only new	2253	Sample Empty	-	-	-	-	-	-	-
B4	beads new	7787	Sample Empty	-	-	-	-	-	-	-
C4	beadsold	951	Sample Empty	-	-	-	-	-	-	-
B2	47-1	7615	Sample Empty	7.8%	6.9%	5.1%	80.1%	23.2%	9.9%	20.6%
B3	47-1d	6894	Sample Empty	8.3%	8.9%	3.9%	78.9%	23.5%	11.0%	20.8%
B1	47-1	5519	Sample Empty	6.1%	7.3%	5.0%	81.6%	26.5%	10.0%	22.2%
C1	47-2	6618	Sample Empty	8.3%	41.7%	12.5%	37.5%	18.9%	11.9%	17.9%
C2	47-2	6409	Sample Empty	33.3%	23.8%	21.4%	21.4%	19.7%	10.4%	18.5%
C3	47-2d	8115	Sample Empty	15.2%	30.3%	27.3%	27.3%	18.3%	11.9%	17.9%
G1	48-2	5122	Sample Empty	8.3%	66.7%	12.5%	12.5%	17.1%	12.5%	17.1%
G2	48-2	5135	Sample Empty	9.8%	43.1%	17.6%	29.4%	16.6%	12.7%	15.0%
G3	48-2d	5012	Sample Empty	9.5%	19.0%	14.3%	57.1%	14.6%	11.5%	15.6%
E1	47-4	5063	Sample Empty	38.1%	4.8%	42.9%	14.3%	30.4%	26.1%	26.1%
A2	48-4	7017	Sample Empty	46.7%	13.3%	10.0%	30.0%	12.5%	18.7%	37.5%
E2	47-4	3109	Sample Empty	14.3%	-14.3%	64.3%	21.4%	40.0%	0.0%	60.0%
A3	48-4	6741	Sample Empty	11.1%	22.2%	16.7%	50.0%	39.0%	7.3%	29.3%
E3	47-4d	6462	Sample Empty	46.7%	13.3%	30.0%	10.0%	44.8%	0.0%	20.7%
A4	48-4d	6426	Sample Empty	26.7%	13.3%	30.0%	30.0%	38.5%	0.0%	23.1%
D1	47-3	4359	Sample Empty	24.2%	30.3%	-9.1%	45.5%	36.4%	18.6%	27.3%
D2	47-3	6637	Sample Empty	33.3%	16.7%	37.5%	12.5%	27.5%	22.5%	37.5%
D3	47-3d	6952	Sample Empty	8.3%	41.7%	37.5%	12.5%	35.5%	29.0%	29.0%

Figure 7.4 Typing table
Percent relative intensity data

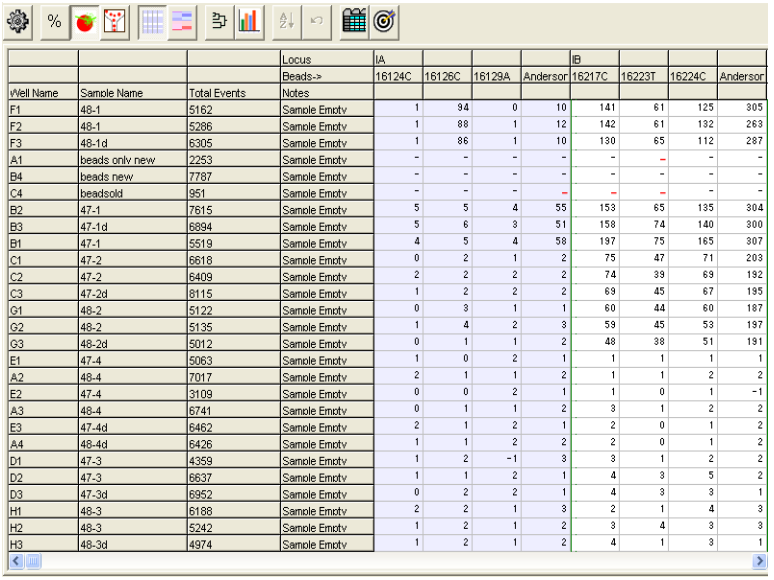
MFI View

In this view, the Typing table shows the background-adjusted MFI of each allele at a particular locus.

- To display the MFI view, click the button.

⇒ The Typing table displays the background-adjusted MFI data of each allele (Figure 7.5).

NOTE: MFI data displayed in red indicates the bead count was less than the user-specified event number set in the Parameter Setting dialog box. A negative MFI value indicates the background value is greater than the MFI.



			Locus	IA				IB			
			Beads->	16124C	16126C	16129A	Andersor	16217C	16223T	16224C	Andersor
Well Name	Sample Name	Total Events	Notes								
F1	48-1	5162	Sample Empty	1	94	0	10	141	61	125	305
F2	48-1	5286	Sample Empty	1	88	1	12	142	61	132	263
F3	48-1d	6305	Sample Empty	1	86	1	10	130	65	112	287
A1	beads only new	2253	Sample Empty	-	-	-	-	-	-	-	-
B4	beads new	7787	Sample Empty	-	-	-	-	-	-	-	-
C4	beadsold	951	Sample Empty	-	-	-	-	-	-	-	-
B2	47-1	7615	Sample Empty	5	5	4	55	153	65	135	304
B3	47-1d	6894	Sample Empty	5	6	3	51	158	74	140	300
B1	47-1	5519	Sample Empty	4	5	4	58	197	75	165	307
C1	47-2	6618	Sample Empty	0	2	1	2	75	47	71	203
C2	47-2	6409	Sample Empty	2	2	2	2	74	39	69	192
C3	47-2d	8115	Sample Empty	1	2	2	2	69	45	67	195
G1	48-2	5122	Sample Empty	0	3	1	1	60	44	60	187
G2	48-2	5135	Sample Empty	1	4	2	3	59	45	53	197
G3	48-2d	5012	Sample Empty	0	1	1	2	48	38	51	191
E1	47-4	5063	Sample Empty	1	0	2	1	1	1	1	1
A2	48-4	7017	Sample Empty	2	1	1	2	1	1	2	2
E2	47-4	3109	Sample Empty	0	0	2	1	1	0	1	-1
A3	48-4	6741	Sample Empty	0	1	1	2	3	1	2	2
E3	47-4d	6462	Sample Empty	2	1	2	1	2	0	1	2
A4	48-4d	6426	Sample Empty	1	1	2	2	2	0	1	2
D1	47-3	4359	Sample Empty	1	2	-1	3	3	1	2	2
D2	47-3	6637	Sample Empty	1	1	2	1	4	3	5	2
D3	47-3d	6952	Sample Empty	0	2	2	1	4	3	3	1
H1	48-3	6188	Sample Empty	2	2	1	3	2	1	4	3
H2	48-3	5242	Sample Empty	1	2	1	2	3	4	3	3
H3	48-3d	4974	Sample Empty	1	2	1	2	4	1	3	1

Figure 7.5 Typing table
Background-adjusted MFI data

Bead Count View

In this view, the Typing table displays the number of events (beads) that the Luminex® system counted for sample acquisition.

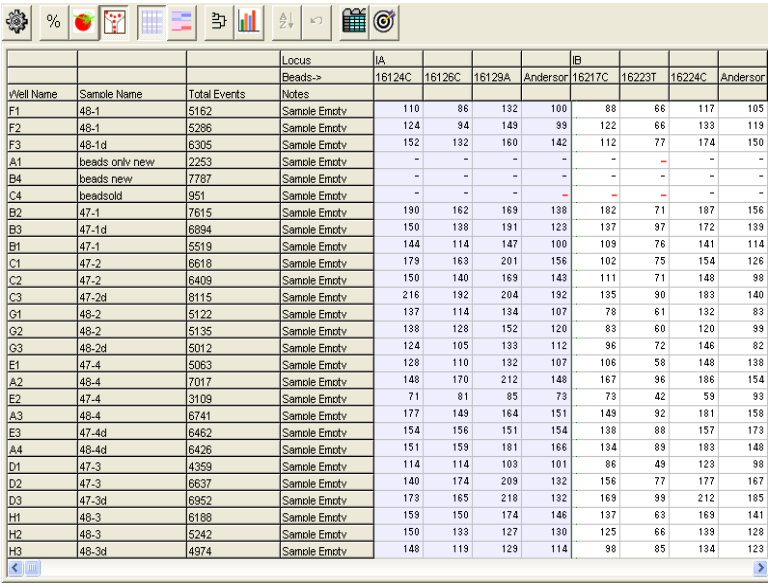
- To display the bead count view, click the button.

  ⇒ The Typing table displays the bead count data for each allele (Figure 7.6).

NOTE: Data displayed in red indicates the bead count was less than the user-specified event number set in the Parameter Setting dialog box.

10. To view the MFI and percent relative intensity data, position the mouse pointer over the table cell of interest.

⇒ A pop-up tool tip displays the MFI and percent relative intensity for the allele.



			Locus	IA				IB			
			Beads->	16124C	16126C	16129A	Andersor	16217C	16223T	16224C	Andersor
Well Name	Sample Name	Total Events	Notes								
F1	48-1	5162	Sample Emotv	110	86	132	100	88	66	117	105
F2	48-1	5286	Sample Emotv	124	94	149	99	122	66	133	119
F3	48-1d	6305	Sample Emotv	152	132	160	142	112	77	174	150
A1	beads only new	2253	Sample Emotv	-	-	-	-	-	-	-	-
B4	beads new	7787	Sample Emotv	-	-	-	-	-	-	-	-
C4	beadsold	951	Sample Emotv	-	-	-	-	-	-	-	-
B2	47-1	7615	Sample Emotv	190	162	169	138	182	71	187	156
B3	47-1d	6894	Sample Emotv	150	138	191	123	137	97	172	139
B1	47-1	5519	Sample Emotv	144	114	147	100	109	76	141	114
C1	47-2	6618	Sample Emotv	179	163	201	156	102	75	154	126
C2	47-2	6409	Sample Emotv	150	140	169	143	111	71	148	98
C3	47-2d	8115	Sample Emotv	216	192	204	192	135	90	183	140
G1	48-2	5122	Sample Emotv	137	114	134	107	78	61	132	83
G2	48-2	5135	Sample Emotv	138	128	152	120	83	60	120	99
G3	48-2d	5012	Sample Emotv	124	105	133	112	96	72	146	82
E1	47-4	5063	Sample Emotv	128	110	132	107	106	58	148	138
A2	48-4	7017	Sample Emotv	148	170	212	148	167	96	186	154
E2	47-4	3109	Sample Emotv	71	81	85	73	73	42	59	93
A3	48-4	6741	Sample Emotv	177	149	164	151	149	92	181	158
E3	47-4d	6462	Sample Emotv	154	156	151	154	138	88	157	173
A4	48-4d	6426	Sample Emotv	151	159	181	166	134	89	183	148
D1	47-3	4359	Sample Emotv	114	114	103	101	86	49	123	98
D2	47-3	6637	Sample Emotv	140	174	209	132	156	77	177	167
D3	47-3d	6952	Sample Emotv	173	165	218	132	169	99	212	185
H1	48-3	6188	Sample Emotv	159	150	174	146	137	63	169	141
H2	48-3	5242	Sample Emotv	150	133	127	130	125	66	139	128
H3	48-3d	4974	Sample Emotv	148	119	129	114	98	85	134	123

Figure 7.6 Typing table
Bead count data

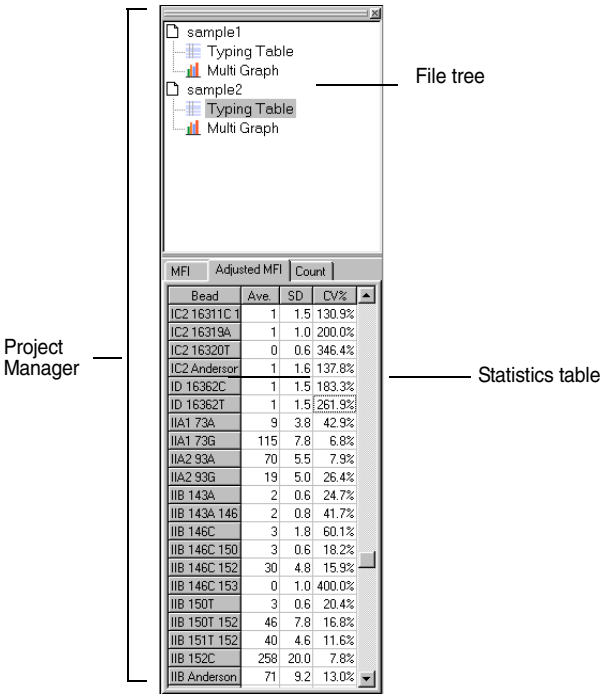
7.2
The Statistics Table

In the Project Manager, the Statistics table displays MFI, background-adjusted MFI, and bead count data for user-specified samples in the Typing table (Figure 7.7). If you select more than one sample, the Statistics table also displays the standard deviation (SD) and coefficient of variation (CV%).

1. Do one of the following to select a sample(s) in the Typing table:
   - Click the sample name.
   - To select adjacent samples (rows), click and hold the mouse while you drag the mouse pointer over the sample names. Click the mouse when the complete selection is highlighted. Alternatively, press and hold the **Shift** key while you click the first and last sample name in the selection.
   - To select nonadjacent samples, press and hold the **Ctrl** key while you click the sample names.

⇒ The Statistics table displays the average MFI for the selected samples, standard deviation (SD), and coefficient of variation (CV%) of the MFI, background adjusted MFI, and bead count data for the selected samples (Figure 7.7).

NOTE: The Statistics table is automatically updated when you select new samples in the Typing table.



Bead	Ave.	SD	CV%
IC2 16311C 1	1	1.5	130.9%
IC2 16319A	1	1.0	200.0%
IC2 16320T	0	0.6	346.4%
IC2 Andersor	1	1.6	137.8%
ID 16362C	1	1.5	183.3%
ID 16362T	1	1.5	261.9%
IIA1 73A	9	3.8	42.9%
IIA1 73G	115	7.8	6.8%
IIA2 93A	70	5.5	7.9%
IIA2 93G	19	5.0	26.4%
IIB 143A	2	0.6	24.7%
IIB 143A 146	2	0.8	41.7%
IIB 146C	3	1.8	60.1%
IIB 146C 150	3	0.6	18.2%
IIB 146C 152	30	4.8	15.9%
IIB 146C 153	0	1.0	400.0%
IIB 150T	3	0.6	20.4%
IIB 150T 152	46	7.8	16.8%
IIB 151T 152	40	4.6	11.6%
IIB 152C	258	20.0	7.8%
IIB Anderson	71	9.2	13.0%

Figure 7.7 Project Manager includes the File tree and Statistics table
Statistics table displays MFI and count data for user-selected samples.

7.3
Sorting Samples in the Typing Table

The Typing table displays the sample data (rows) in the order that the data were collected in the Luminex® system. You can sort samples by sample name (alpha-numeric sort) or by similarity to the expression level (MFI data) of a user-specified sample.

Sorting by Sample Name

- To sort the Typing table by sample name, right-click the name that you want to use for the reference, and select **Sort by Sample → Ascending** (or **Descending**) from the shortcut menu that appears (Figure 7.8).

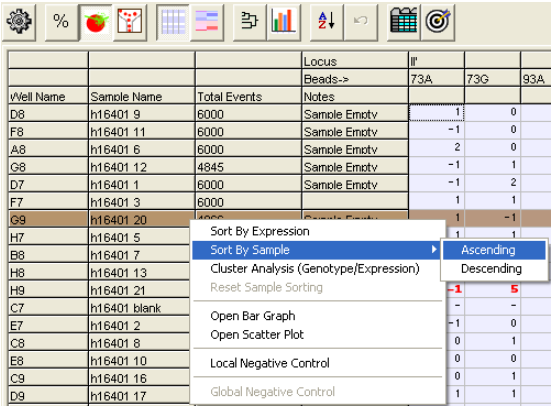
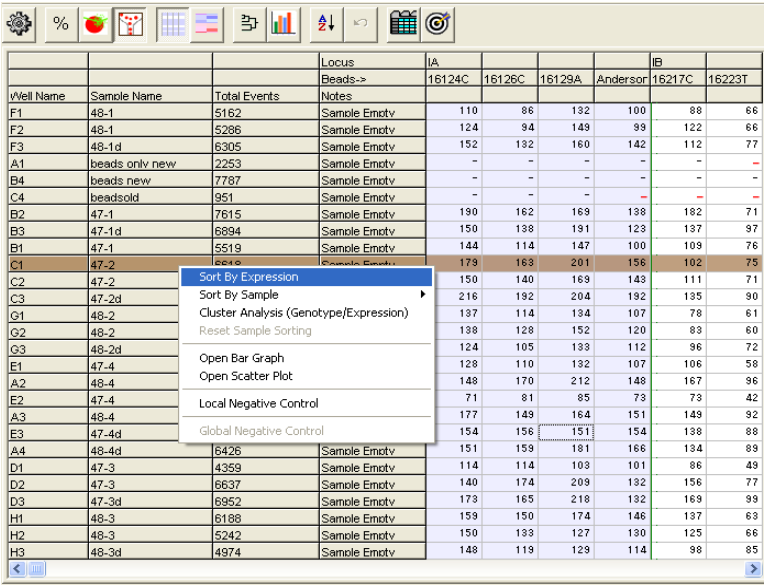


Figure 7.8 Typing table
Sorting by sample name (alpha-numeric sort).

Sorting by Expression Level

1. Do either of the following to sort the Typing table by expression level:
   - Right-click the sample name that you want to use as the reference for the sort and click **Sort By Expression Level** in the shortcut menu that appears (Figure 7.9).
   - Click the sample name that you want to use as the reference for the sort, then click the **Sort** button .

   ⇒ The Typing table displays the reference sample in the first row and sorts the remaining samples (rows) by similar expression level in descending order.



			Locus	IA				IB	
			Beads->	16124C	16126C	16129A	Andersor	16217C	16223T
Well Name	Sample Name	Total Events	Notes						
F1	48-1	5162	Sample Empty	110	86	132	100	88	66
F2	48-1	5286	Sample Empty	124	94	149	99	122	66
F3	48-1d	6305	Sample Empty	152	132	160	142	112	77
A1	beads only new	2253	Sample Empty	-	-	-	-	-	-
B4	beads new	7787	Sample Empty	-	-	-	-	-	-
C4	beadsold	951	Sample Empty	-	-	-	-	-	-
B2	47-1	7615	Sample Empty	190	162	169	138	182	71
B3	47-1d	6894	Sample Empty	150	138	191	123	137	97
B1	47-1	5519	Sample Empty	144	114	147	100	109	76
C1	47-2	[illegible]	[illegible]	179	163	201	156	102	75
C2	47-2	[illegible]	[illegible]	150	140	169	143	111	71
C3	47-2d	[illegible]	[illegible]	216	192	204	192	135	90
G1	48-2	[illegible]	[illegible]	137	114	134	107	78	61
G2	48-2	[illegible]	[illegible]	138	128	152	120	83	60
G3	48-2d	[illegible]	[illegible]	124	105	133	112	96	72
E1	47-4	[illegible]	[illegible]	128	110	132	107	106	58
A2	48-4	[illegible]	[illegible]	148	170	212	148	167	96
E2	47-4	[illegible]	[illegible]	71	81	85	73	73	42
A3	48-4	[illegible]	[illegible]	177	149	164	151	149	92
E3	47-4d	[illegible]	[illegible]	154	156	151	154	138	88
A4	48-4d	6426	Sample Empty	151	159	181	166	134	89
D1	47-3	4359	Sample Empty	114	114	103	101	86	49
D2	47-3	6637	Sample Empty	140	174	209	132	156	77
D3	47-3d	6952	Sample Empty	173	165	218	132	169	99
H1	48-3	6188	Sample Empty	159	150	174	146	137	63
H2	48-3	5242	Sample Empty	150	133	127	130	125	66
H3	48-3d	4974	Sample Empty	148	119	129	114	98	85

Figure 7.9 Typing table
Sorting by expression level (MFI data).

2. To view the homology score for a sample, position the mouse pointer over the sample name.
   ⇒ A pop-up tool tip displays the sample name and homology score.

Resetting the Sample Sort

To reset the Typing table sample rows to the default (the order in which the data were collected in the Luminex® system), do either of the following:

- Click the **Reset Sample Sort** button .
- Right-click a sample row and click **Reset Sample Sorting** in the shortcut menu that appears.

⇒ The Typing table displays the sample rows in the order that the data were collected in the Luminex system.

7.4
Allele Call Table

The Allele Call table displays the alleles called for each sample (Figure 7.10). In the Allele Call table you can:

- view genotype or haplotype
- sort samples by homology to a user-selected reference sample
- view allele frequency, locus (group) frequency, or genotype frequency

1. To view the Allele Call table, open the project of interest and do one of the following:
   - click the  button
   - right-click the Typing table and click **Allele Call** in the shortcut menu that appears
   - select **Function → Allele Call** from the menu bar

   ⇒ A separate window opens and displays the Allele Call table (Figure 7.10).

   The alleles are highlighted using the group or allele color (specified in the Parameter Setting dialog box, see *Group and Allele Color* on page 6.8).

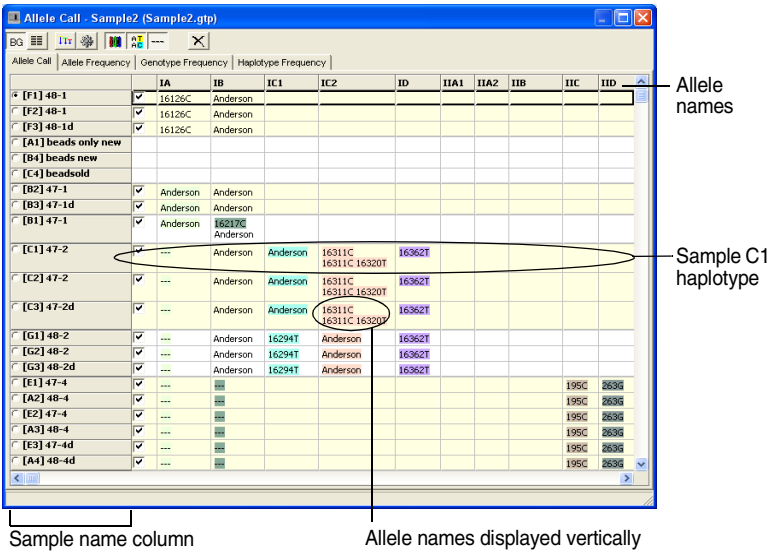


Figure 7.10 Allele Call table
Genotype call displayed vertically.

2. If you want to display multiple allele calls horizontally, click the button.
   ⇒ The allele names are displayed side-by-side (Figure 7.11).

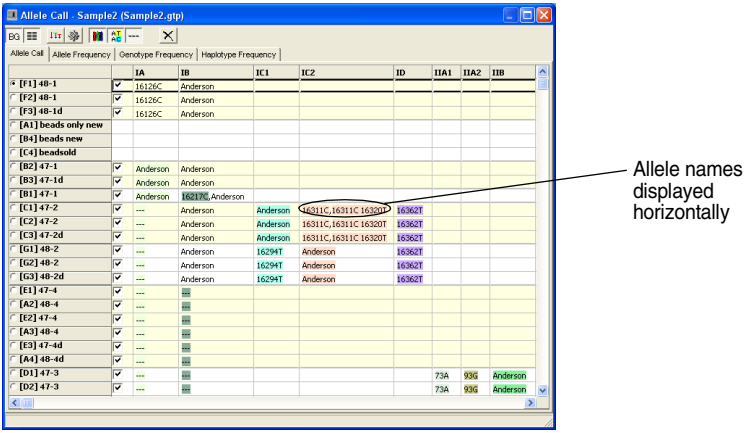


Figure 7.11 Allele Call table
Genotype call displayed horizontally.

3. To copy the current view of the Allele Call table, right-click the table and select **Copy Table as Text** or **Copy Table as Text (Without Quotations)** from the shortcut menu that appears.
   ⇒ The table information is copied to the system clipboard.
4. To print the current view of the Allele Call table, right-click the table and select **Print Allele Call Table** from the shortcut menu that appears.
5. Click the Close button ✕ to close the Allele Call table.

Merging Wells

Sometimes an assay format stipulates the same sample in several different wells and probes each well with a different bead set. In the Allele Call table, you can merge the different wells and view the complete genotype results across all wells for the sample (Figure 7.12).

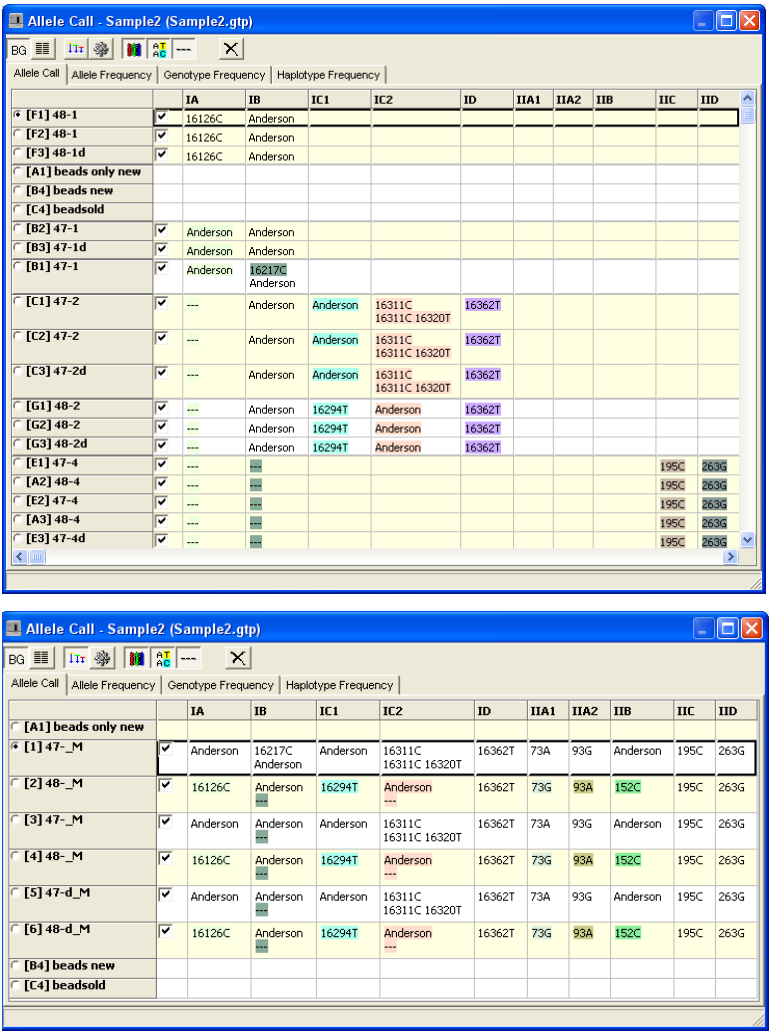


Figure 7.12 Allele Call table, before a well merge (top) and after a well merge (bottom)

1. In the Allele Call table, click the **Open Well Merge** button .
   ⇒ The Well Merge dialog box appears (Figure 7.13).

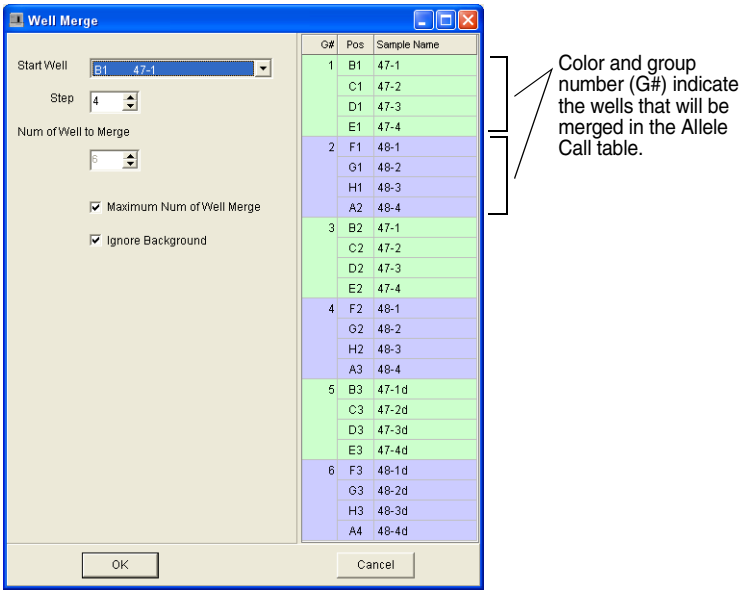


Figure 7.13 Well Merge dialog box

2. In the Start Well drop-down list, select the first well for the merge.
3. In the Step box, enter the total number of wells to include in the merge.

   ⇒ The group number, well position, and sample names included in the merge are updated (Figure 7.13).
4. Choose the **Maximum Num of Well Merge** option to create the maximum number of groups using the step number (entered in step 3).
5. Choose the **Ignore Background** option to omit background wells from the merge.

NOTE: If the **Background Hidden** option is enabled, the **Ignore Background** option is not available.

6. Click **OK**.

   ⇒ The wells are merged in the Allele Call table (Figure 7.12).
7. To toggle the Allele Call table between the pre- and post-merge views, click the **Toggle Normal/Merged Well** button .

Allele Call Table Viewing Options

Selecting a Reference Sample

You can specify a reference sample in the Allele Call table. Two viewing options are available: you can paint (highlight) the alleles that are called the same as the reference sample, or you can paint the alleles that are called different from the reference sample.

1. In the Allele Call table, make sure the Reference Sample Selection radio buttons are displayed (Figure 7.14). If the radio buttons are not displayed, click the button to display them.

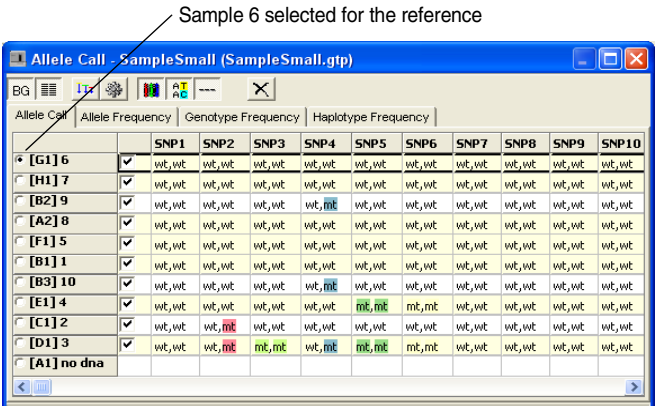


Figure 7.14 Allele Call table
Alleles called different from the reference (sample 6) are painted.

2. Click the radio button next to the sample you want to use as the reference.
   ⇒ Alleles that are called different from the reference are painted using the group or allele color, depending on what is selected in the Parameter Setting dialog box. (See *Group and Allele Color* on page 6.8).
3. To toggle the view and paint the alleles that are called the same as the reference, click the button (Figure 7.15).

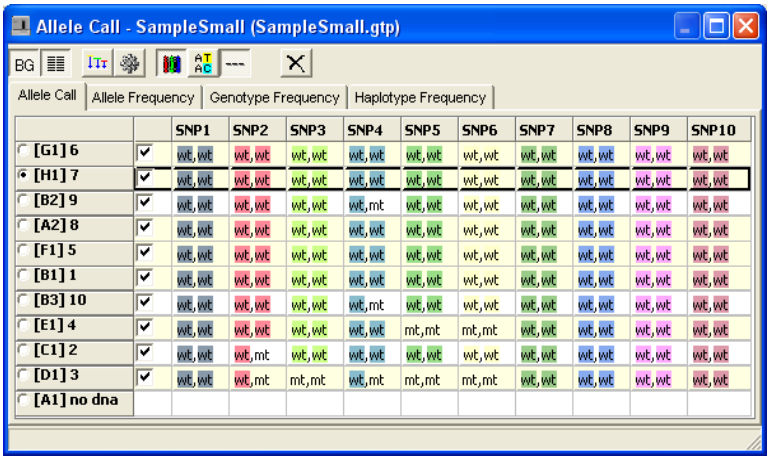


Figure 7.15 Allele Call table
Alleles called the same as sample 6 (user-selected reference) are painted.

4. To indicate samples that have fewer alleles called at a locus than the reference sample, click the --- button.
   ⇒ The Allele Call table displays '---' for samples that have fewer alleles called at a locus than the reference sample (Figure 7.16).

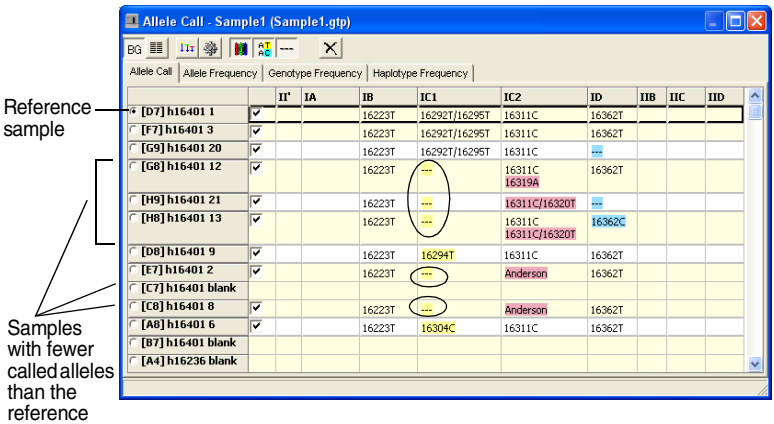


Figure 7.16 Allele Call table
Samples that have fewer alleles called at a locus than the reference sample display '---'.

Sorting Samples by Expression Level

You can sort samples in the Allele Call table by expression level (MFI data).

1. In the sample column of the Allele Call table, right-click the sample you want to use as the reference sample for the sort and click **Sort By Expression Level** in the shortcut menu that appears (Figure 7.17).

   ⇒ The Allele Call table displays the reference sample in the top row and sorts the remaining samples by expression level in descending order (top to bottom).

   After the sort, allele calls different from the reference sample are painted (highlighted) with the group or allele color (Figure 7.17). (These colors are specified in the Parameter Setting dialog box, see page 6.8). Allele calls that are the same as the reference sample are not painted.

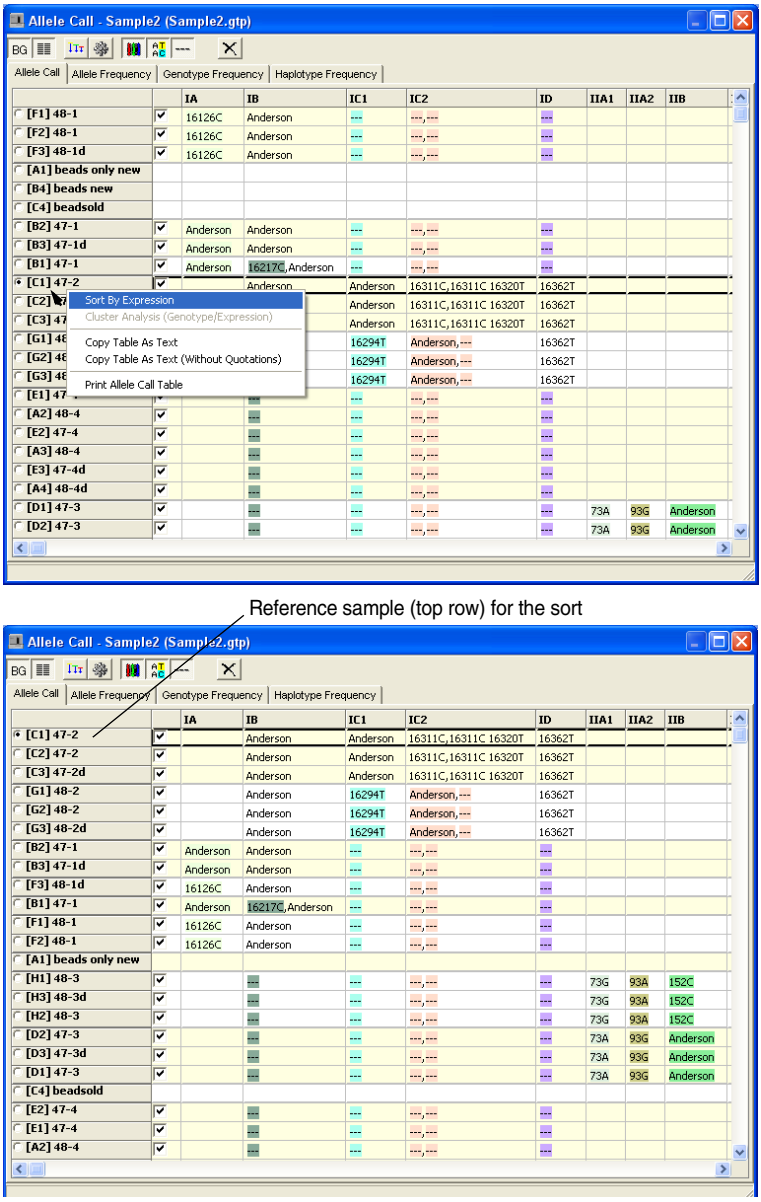


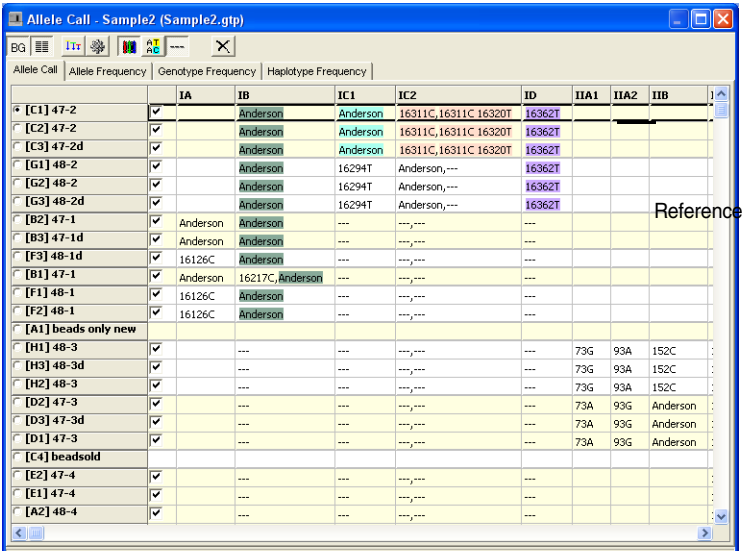
Figure 7.17 Allele Call table sorted by expression

Choose a reference sample (top). After the sort, the reference sample appears in the stop row and allele calls are sorted in descending order of expression; calls that differ from the reference are painted (bottom).

2. To toggle the view and paint (highlight) the allele calls that are the same as the reference sample, click the button.

   ⇒ Allele calls that are the same as the reference sample are painted with the group or allele color (specified in the Parameter Setting dialog box, see *Group and Allele Color* on page 6.8). Allele calls that are different from the reference sample are not painted (Figure 7.18).

.



		IA	IB	IC1	IC2	ID	IIA1	IIA2	IIB
[C1] 47-2	✓		Anderson	Anderson	16311C,16311C 16320T	16362T			
[C2] 47-2	✓		Anderson	Anderson	16311C,16311C 16320T	16362T			
[C3] 47-2d	✓		Anderson	Anderson	16311C,16311C 16320T	16362T			
[G1] 48-2	✓		Anderson	16294T	Anderson,---	16362T			
[G2] 48-2	✓		Anderson	16294T	Anderson,---	16362T			
[G3] 48-2d	✓		Anderson	16294T	Anderson,---	16362T			
[B2] 47-1	✓	Anderson	Anderson	---	---,---	---			
[B3] 47-1d	✓	Anderson	Anderson	---	---,---	---			
[F3] 48-1d	✓	16126C	Anderson	---	---,---	---			
[B1] 47-1	✓	Anderson	16217C,Anderson	---	---,---	---			
[F1] 48-1	✓	16126C	Anderson	---	---,---	---			
[F2] 48-1	✓	16126C	Anderson	---	---,---	---			
[A1] beads only new									
[H1] 48-3	✓		---	---	---,---	---	73G	93A	152C
[H3] 48-3d	✓		---	---	---,---	---	73G	93A	152C
[H2] 48-3	✓		---	---	---,---	---	73G	93A	152C
[D2] 47-3	✓		---	---	---,---	---	73A	93G	Anderson
[D3] 47-3d	✓		---	---	---,---	---	73A	93G	Anderson
[D1] 47-3	✓		---	---	---,---	---	73A	93G	Anderson
[C4] beadsold									
[E2] 47-4	✓		---	---	---,---	---			
[E1] 47-4	✓		---	---	---,---	---			
[A2] 48-4	✓		---	---	---,---	---			

Figure 7.18 Allele Call table
Allele calls sorted by expression level. Allele calls the same as the reference are painted.

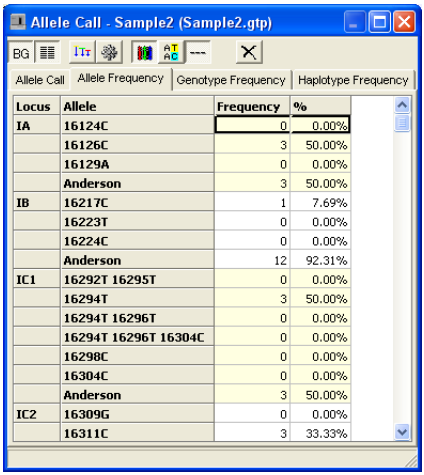
Allele Frequency

The allele frequency for a sample is:

> Number of a particular allele call/Total number of allele calls in the sample

1. To view allele frequency, click the Allele Frequency tab in the Allele Call table (Figure 7.19).
2. To copy the allele frequency information, right-click the table and click **Copy Table as Text** from the shortcut menu that appears.

   ⇒ The table information is copied to the system clipboard.



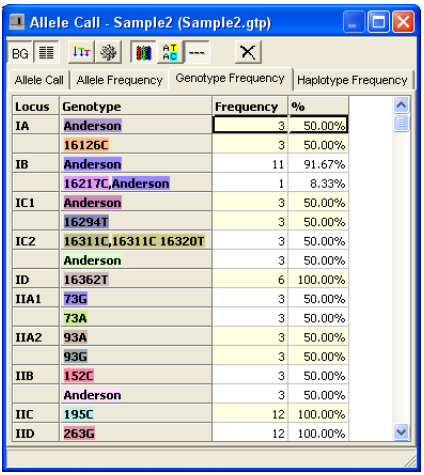
Locus	Allele	Frequency	%
IA	16124C	0	0.00%
	16126C	3	50.00%
	16129A	0	0.00%
	Anderson	3	50.00%
IB	16217C	1	7.69%
	16223T	0	0.00%
	16224C	0	0.00%
	Anderson	12	92.31%
IC1	16292T 16295T	0	0.00%
	16294T	3	50.00%
	16294T 16296T	0	0.00%
	16294T 16296T 16304C	0	0.00%
	16298C	0	0.00%
	16304C	0	0.00%
	Anderson	3	50.00%
IC2	16309G	0	0.00%
	16311C	3	33.33%

Figure 7.19 Allele Call table, Allele Frequency tab

Genotype Frequency

The Genotype Frequency tab (Figure 7.20) displays the frequency and percentage for each allele or combination of alleles called at each locus (group).

1. To view the genotype frequency information, click the Genotype Frequency tab.
2. To copy the genotype frequency information, right-click the table and click **Copy Table as Text** from the shortcut menu that appears.
   ⇒ The table information is copied to the system clipboard.



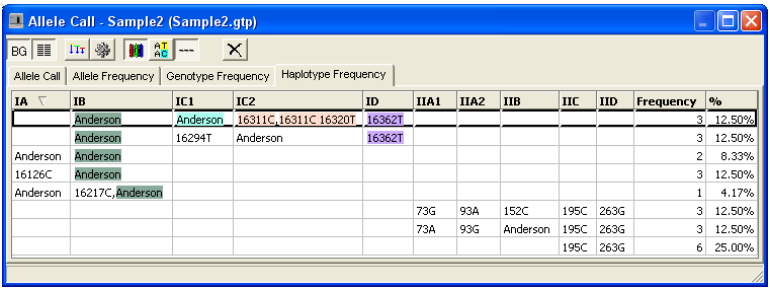
Locus	Genotype	Frequency	%
IA	Anderson	3	50.00%
	16126C	3	50.00%
IB	Anderson	11	91.67%
	16217C,Anderson	1	8.33%
IC1	Anderson	3	50.00%
	16294T	3	50.00%
IC2	16311C,16311C 16320T	3	50.00%
	Anderson	3	50.00%
ID	16362T	6	100.00%
IIA1	73G	3	50.00%
	73A	3	50.00%
IIA2	93A	3	50.00%
	93G	3	50.00%
IIB	152C	3	50.00%
	Anderson	3	50.00%
IIC	195C	12	100.00%
IID	263G	12	100.00%

Figure 7.20 Allele Call table, Genotype Frequency tab

Haplotype Frequency

The haplotype frequency tab (Figure 7.21) displays the frequency and percentage for the genotypes that were called.

1. To view the haplotype frequency information, click the Haplotype Frequency tab.
2. To copy the haplotype frequency information, right-click the table and click **Copy Table as Text** from the shortcut menu that appears.
   ⇒ The table information is copied to the system clipboard.



IA	IB	IC1	IC2	ID	IIA1	IIA2	IIB	IIC	IID	Frequency	%
	Anderson	Anderson	16311C,16311C 16320T	16362T						3	12.50%
	Anderson	16294T	Anderson	16362T						3	12.50%
Anderson	Anderson									2	8.33%
16126C	Anderson									3	12.50%
Anderson	16217C,Anderson									1	4.17%
					73G	93A	152C	195C	263G	3	12.50%
					73A	93G	Anderson	195C	263G	3	12.50%
								195C	263G	6	25.00%

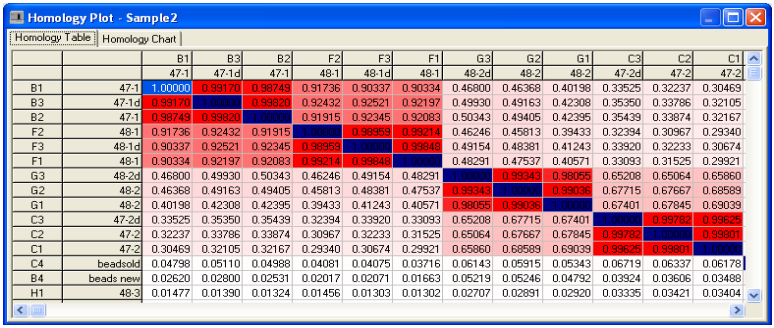
Figure 7.21 Allele Call table, Haplotype Frequency tab

7.5
Homology Table and Chart

The MasterPlex™ GT software computes a homology score for each pair of samples in the Typing table. It applies a least squares analysis to the expression level of the alleles (MFI data) for each sample pair. The Homology table displays the homology scores for the sample pairs (Figure 7.22) and the Homology chart plots the data in 3-dimensional space (Figure 7.23).

1. To view the Homology table, click the  button.
   ⇒ A separate window opens and displays the Homology table (Figure 7.22).

   In the Homology table, the scores are colored: white (no homology = 0), blue (perfect homology = 1), and shades of red (a darker shade represents a larger homology score).



		B1	B3	B2	F2	F3	F1	G3	G2	G1	C3	C2	C1
		47-1	47-1d	47-1	48-1	48-1d	48-1	48-2d	48-2	48-2	47-2d	47-2	47-2
B1	47-1	1.00000	0.99170	0.98749	0.91736	0.90337	0.90334	0.46800	0.46368	0.40198	0.33525	0.32237	0.30469
B3	47-1d	0.99170	1.00000	0.99820	0.92432	0.92521	0.92197	0.49930	0.49163	0.42308	0.35350	0.33786	0.32105
B2	47-1	0.98749	0.99820	1.00000	0.91915	0.92345	0.92083	0.50343	0.49405	0.42395	0.35439	0.33874	0.32167
F2	48-1	0.91736	0.92432	0.91915	1.00000	0.98953	0.99214	0.46246	0.45813	0.39433	0.32394	0.30967	0.29340
F3	48-1d	0.90337	0.92521	0.92345	0.98959	1.00000	0.99848	0.49154	0.48381	0.41243	0.33920	0.32233	0.30674
F1	48-1	0.90334	0.92197	0.92083	0.99214	0.99848	1.00000	0.48291	0.47537	0.40571	0.33093	0.31525	0.29921
G3	48-2d	0.46800	0.49930	0.50343	0.46246	0.49154	0.48291	1.00000	0.99343	0.98055	0.65208	0.65064	0.65860
G2	48-2	0.46368	0.49163	0.49405	0.45813	0.48381	0.47537	0.99343	1.00000	0.99036	0.67715	0.67667	0.68589
G1	48-2	0.40198	0.42308	0.42395	0.39433	0.41243	0.40571	0.98055	0.99036	1.00000	0.67401	0.67845	0.69039
C3	47-2d	0.33525	0.35350	0.35439	0.32394	0.33920	0.33093	0.65208	0.67715	0.67401	1.00000	0.99782	0.99625
C2	47-2	0.32237	0.33786	0.33874	0.30967	0.32233	0.31525	0.65064	0.67667	0.67845	0.99782	1.00000	0.99801
C1	47-2	0.30469	0.32105	0.32167	0.29340	0.30674	0.29921	0.65860	0.68589	0.69039	0.99625	0.99801	1.00000
C4	beadsold	0.04798	0.05110	0.04988	0.04081	0.04075	0.03716	0.06143	0.05915	0.05343	0.06719	0.06337	0.06178
B4	beads new	0.02620	0.02800	0.02531	0.02017	0.02071	0.01663	0.05219	0.05246	0.04792	0.03924	0.03606	0.03488
H1	48-3	0.01477	0.01390	0.01324	0.01456	0.01303	0.01302	0.02707	0.02891	0.02920	0.03335	0.03421	0.03404

Figure 7.22 Homology table

2. To copy the Homology table, right-click the table and select **Copy Table As Text** from the shortcut menu that appears.
3. To view the Homology chart, click the Homology Chart tab.
   ⇒ The window displays the Homology chart (Figure 7.23).

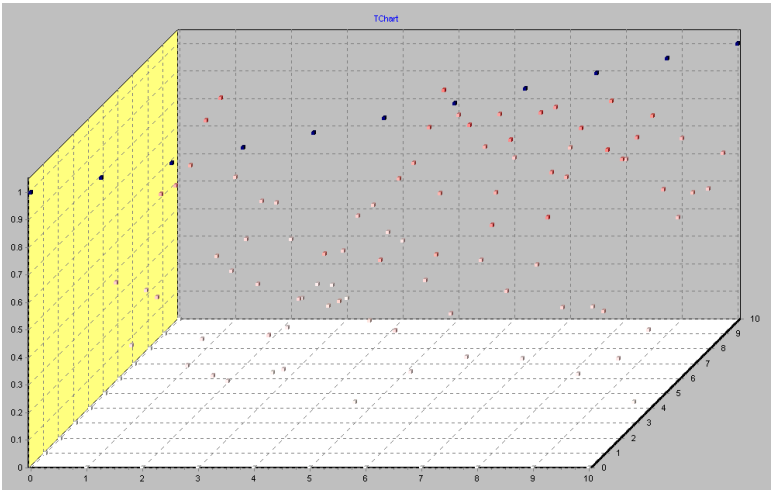


Figure 7.23 Homology chart

4. To view information about a point in the chart, position the mouse pointer over the point.

   ⇒ A pop-up tool tip displays the sample names and homology score.
5. To change the 3-dimensional view of the chart, click and hold the mouse while you drag the pointer. To reset the view, right-click the chart and click **Reset 3D View** from the shortcut menu that appears.
6. To copy the Homology chart, right-click the chart and select **Copy As a Bitmap** or **Copy As Windows Meta Format** from the shortcut menu that appears.
7. To add the Homology chart to a report, right-click the chart and select **Add To Report** from the shortcut menu that appears.

7.6
Viewing Graphs for Selected Samples

You can select samples in the Typing table and view the data in the Multi Compare bar graph, Depth bar graph, or Sample scatter plot. (See *Graphs* on page 8.1 for more information.)

Multi Compare and Depth Bar Graph

The Multi Compare bar graph displays background adjusted MFI (Figure 7.24). The graphs for the selected samples are tiled horizontally to help you compare samples and distinguish differences.

The Depth bar graph plots the background adjusted MFI or RI for all selected samples in one bar graph (Figure 7.25). (See page 8.6 and page 8.8 for more information about the Multi Compare and Depth bar graphs.)

1. Do one of the following to select samples in the Typing table for the graphs:
   - To select adjacent samples (columns), click and hold the mouse while you drag the mouse pointer over the sample names (column headers). Click the mouse when you complete the selection. Alternatively, press and hold the **Shift** key while you click the first and last sample name in the selection.
   - To select nonadjacent samples, press and hold the **Ctrl** key while you click the sample names.
2. Right-click a selected sample name and select **Open Bar Graph** from the shortcut menu that appears.

   ⇒ The Multi Graph view appears (Figure 7.24).
3. Click the Multi Compare tab to view a separate bar graph of MFI or RI data for each selected sample (Figure 7.24).
4. Click the Depth tab to display the Depth bar graph (Figure 7.25).
5. To return to the Typing table for the active results, click the  button.

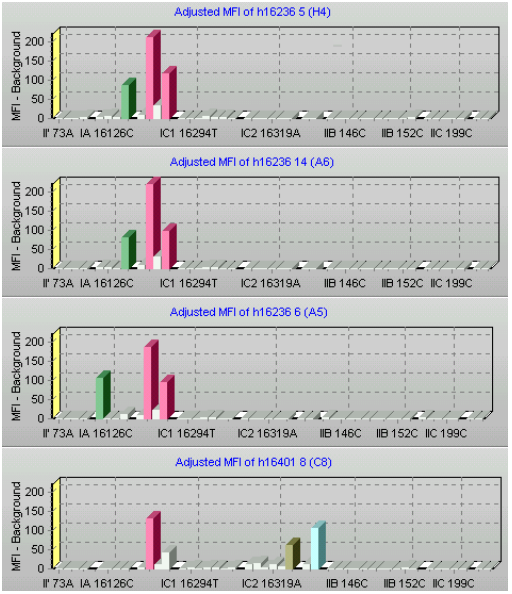


Figure 7.24 Multi Compare bar graphs (four samples)

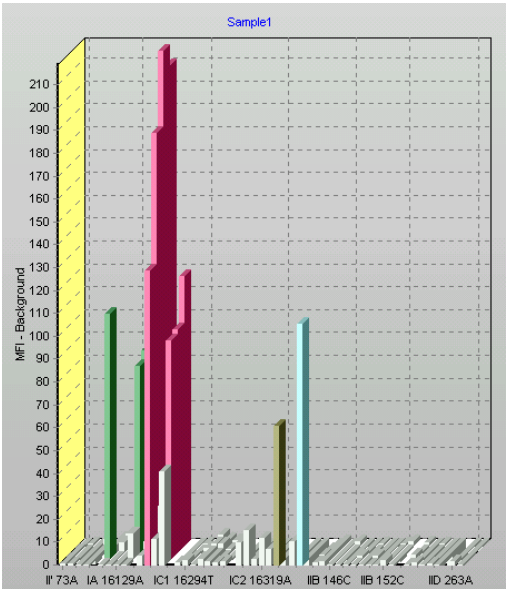


Figure 7.25 Depth bar graph (for the four samples in Figure 7.15)

Sample by Sample Scatter Graph

The Sample by Sample scatter graph plots the background adjusted MFI for two user-selected samples. Each point in the graph represents an allele. (For more information, see *Sample by Sample Scatter Graph* on page 8.19.)

1. Right-click a sample you want to plot in the scatter graph, and select **Sort by Expression** from the shortcut menu.

   ⇒ This places the selected sample in the left column of the Typing table and sorts the Typing table by expression level.

2. Right-click the second sample for the scatter plot and select **Open Scatter Plot** from the shortcut menu.

   ⇒ The Sample scatter graph is displayed (Figure 7.26).

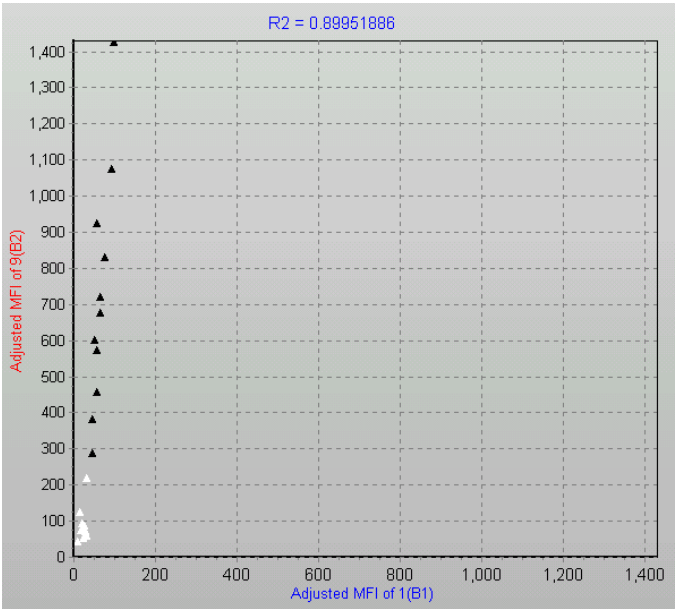


Figure 7.26 Sample scatter graph

NOTE: Opening the Sample by Sample scatter graph puts the Multi Graph view in *Two Sample Mode* (only the top sample in the Sample Name list and one other user-selected sample can be displayed in the Multi Graph view). To exit this mode when you are done viewing the Sample by Sample scatter graph, click the button.

The MasterPlex™ GT software can plot the MFI data in the following graphical formats:

- Multi Compare bar graph
- Depth bar graph
- Sample by Sample scatter plot
- Allele by Allele scatter plot
- Heat map

This chapter explains how to work in the Multi Graph view.

8.1 The Multi Graph View

To display the Multi Graph view for:

- the active results, click the  button
- a particular project in the Project Manager, click **Multi Graph** under the file of interest in the file tree Figure 8.1

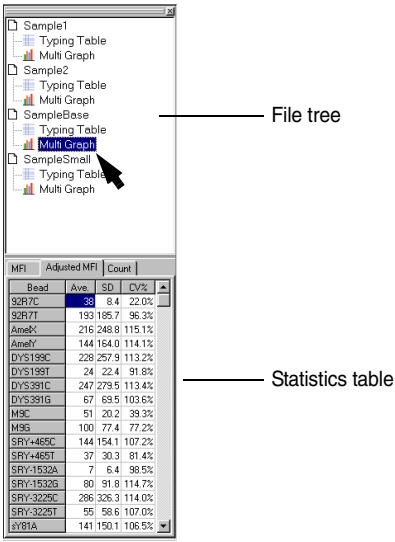


Figure 8.1 Project Manager

In the Multi Graph view (Figure 8.2), the sample list displays the samples in the active results. The project manager displays MFI and count data for samples that are highlighted in the sample list.

The Multi Graph view displays each type of graph in a separate tab. Table 8.1 provides a brief summary of each graph type.

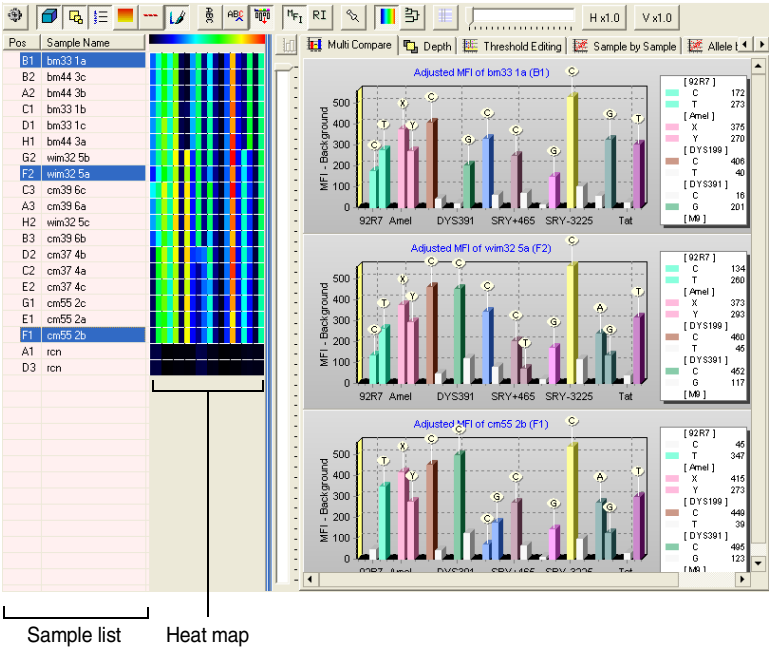


Figure 8.2 Multi Graph view
Heat map and Multi Compare bar graphs for three user-selected samples.

Table 8.1 MasterPlex™ GT graphs

Graph Type	Displays a...
Multi Compare Graph (Figure 8.2)	Bar graph of background-adjusted median fluorescence intensity (MFI) or relative intensity (RI) values for each user-selected sample.
Depth Graph (Figure 8.7)	Composite bar graph of background adjusted MFI or RI data for all user-selected samples.
Sample by Sample Scatter Graph (Figure 8.15)	Scatter plot of background adjusted MFI data for a user-selected pair of samples. Each point represents an allele.
Allele by Allele Scatter Graph (Figure 8.17)	Scatter plot of background adjusted MFI data for user-selected pairs of alleles. Each point represents a sample.
Heat Map (Figure 8.3)	Color-coded representation of the MFI data for each sample.

Sorting Samples by Expression Level

In the Multi Graph view, you can sort the sample list by expression level (MFI data). This is useful for comparing and choosing samples for the graphs.

1. In the sample list Figure 8.2, right-click the sample you want to use as the reference for the sort.
2. Click **Sort By Expression** in the shortcut menu that appears.
   ⇒ The user-selected sample is displayed at the top of the sample list, and the remaining samples are sorted by similar expression level (descending order).

Resetting the Sort

To reset the sample list to the default sort (the order that the data were collected by the Luminex® system), right-click the sample list and click **Reset Sample Sorting** from the shortcut menu that appears.

This also resets the sample sort in the Typing table.

8.2
Heat Map

The Heat map is a color-coded representation of background-adjusted MFI data for each sample. The color range from black (low) to red (high) represents the allele expression level. The Heat map provides a convenient way to quickly compare the expression level of the alleles in a single sample as well as the expression level of a single allele across multiple samples.

The map rows organize the alleles (from left to right) in the same order as the Statistics table (from top to bottom) (Figure 8.3). A map represents one sample and shows the expression level of each allele in the sample. Each column in the map represents one allele and shows the expression level for that allele across all of the samples.

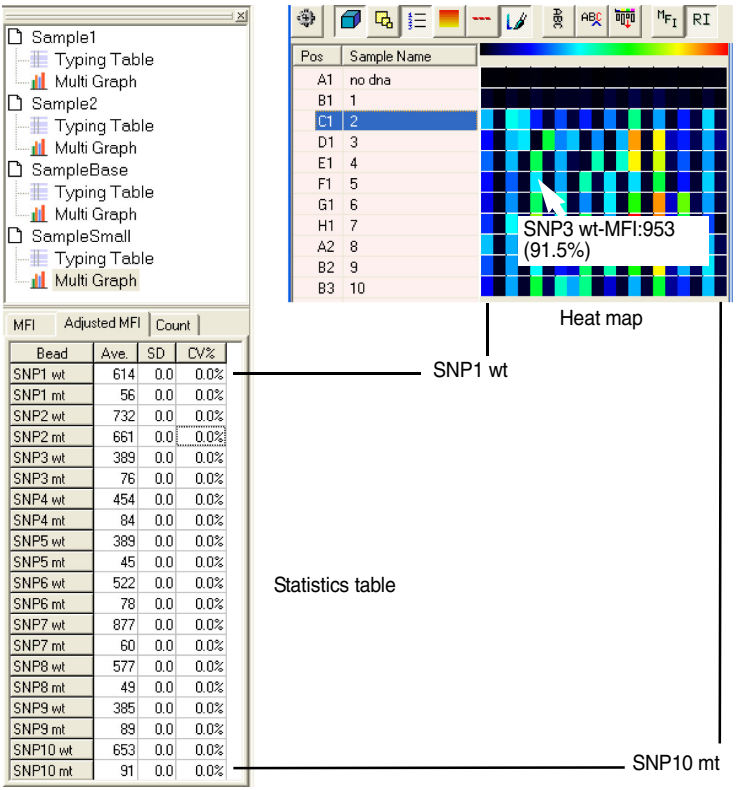


Figure 8.3 Heat map
Alleles from left to right.

1. To show or hide the Heat map, click the toolbar button.
2. To view allele MFI data, position the mouse pointer over the allele of interest in the Heat map.

   ⇒ A pop-up tool tip shows the allele name, background-adjusted MFI data, and relative intensity data Figure 8.3.

3. To change the width of the bars in the map, open the Applications Options dialog box (select **Option Set →Application Options** from the menu bar) and enter a pixel number for the bar size (minimum = 1 pixel/allele, maximum = 10 pixels/allele) (Figure 8.4).

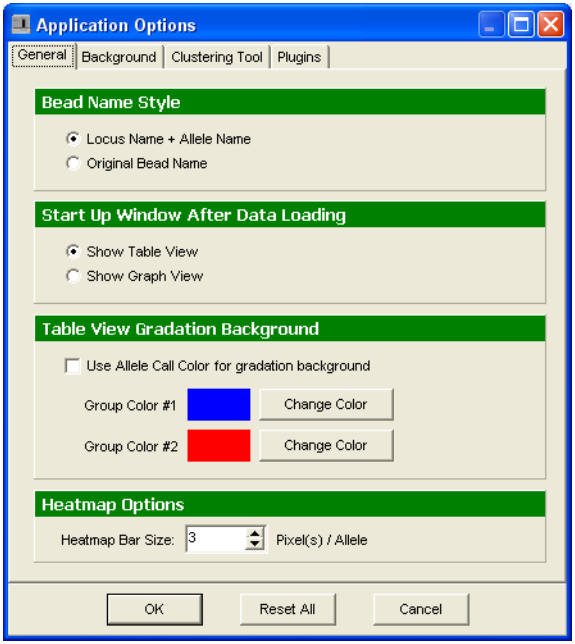


Figure 8.4 Application Options dialog box

8.3 Multi Compare Graph

The Multi Compare bar graph displays the background-adjusted MFI or RI data for user-selected samples in a bar graph format. It is a useful way to view the sample genotype (or haplotype) and compare expression levels across samples.

1. Open the Multi Graph view for the results you want to graph.
2. In the Sample Name list, highlight each sample that you want to display in a Multi Compare graph Figure 8.5.

   To select adjacent samples, press and hold the **Shift** key while you click the first and last sample in the selection. To select nonadjacent samples, press and hold the **Control** key while you click the samples.

   ⇒ A Multi Compare graph displays background adjusted MFI data for each selected sample ((Figure 8.5)).

3. To display relative intensity (RI) data (Figure 8.6), click the RI button.
4. To display allele information, put the mouse pointer over a bar.

   ⇒ A pop-up tool tip displays the allele name and intensity data (Figure 8.5).

5. To clear the Multi Compare bar graphs, click an empty row in the sample list.

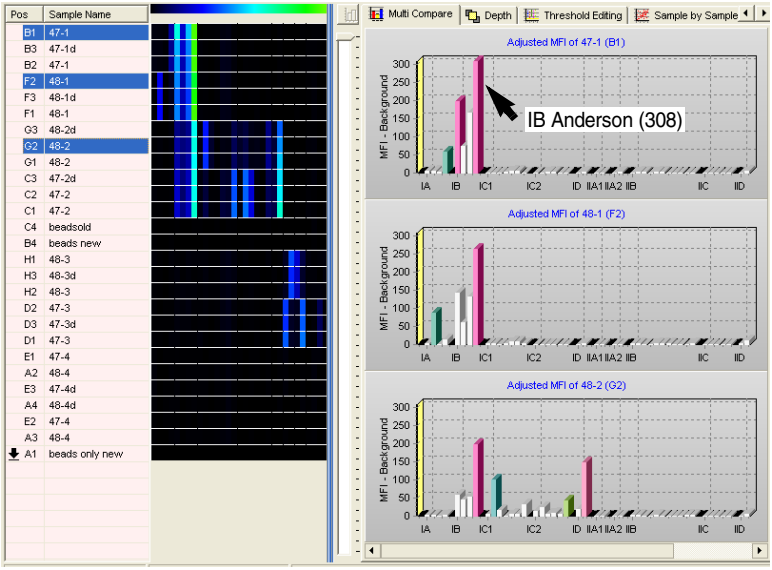


Figure 8.5 Multi Compare graphs
MFI data

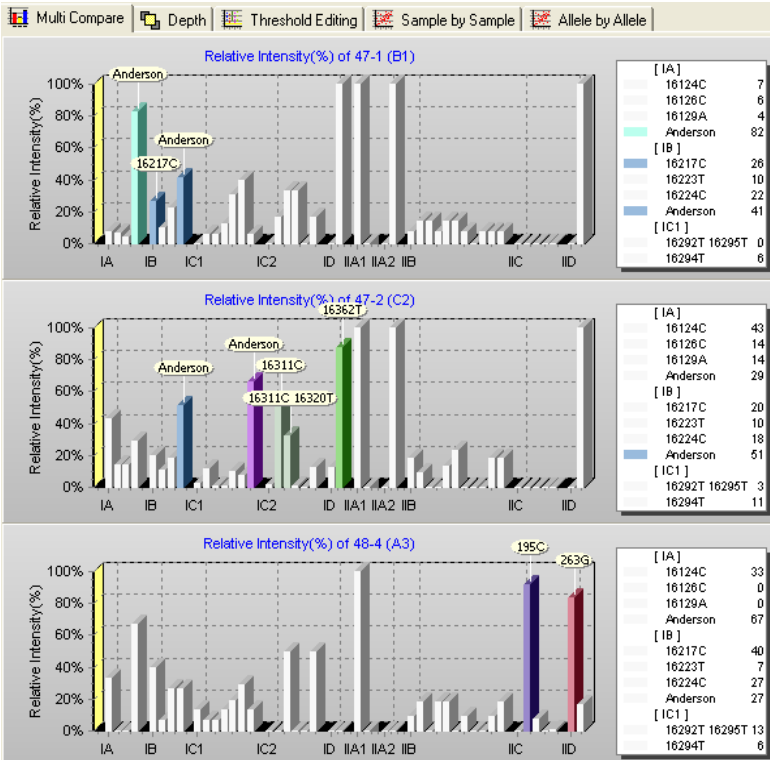


Figure 8.6 Multi Compare graphs
Relative intensity data

8.4
Depth Graph

The Depth graph plots the expression profiles (background-adjusted MFI or RI data) for user-selected samples in one bar graph. It is a useful way to compare allele expression levels across samples.

1. Open the Multi Graph view for the results you want to graph and click the Depth tab.
2. In the Sample Name list (Figure 8.7), highlight the samples you want to display in the Depth graph.

   To select adjacent samples, press and hold the **Shift** key while you click the first and last sample in the selection. To select nonadjacent

samples, press and hold the **Control** key while you click the samples.

⇒ The Depth graph displays the background-adjusted MFI data for the selected samples (Figure 8.7).

3. To display allele information, put the mouse pointer over a bar.

   ⇒ A pop-up tool tip displays the allele name and intensity data (Figure 8.7).

4. To display relative intensity (RI) data Figure 8.8, click the RI button.
5. To rotate the 3-dimensional (3D) view of the Depth graph, click and hold the mouse while you move the mouse pointer in a horizontal or vertical direction.

   ⇒ The graph view rotates horizontally or vertically.

6. To reset the 3D view, right-click the graph and select **Reset 3D View** in the shortcut menu that appears.
7. To clear the Depth graph, click an empty row in the sample list.

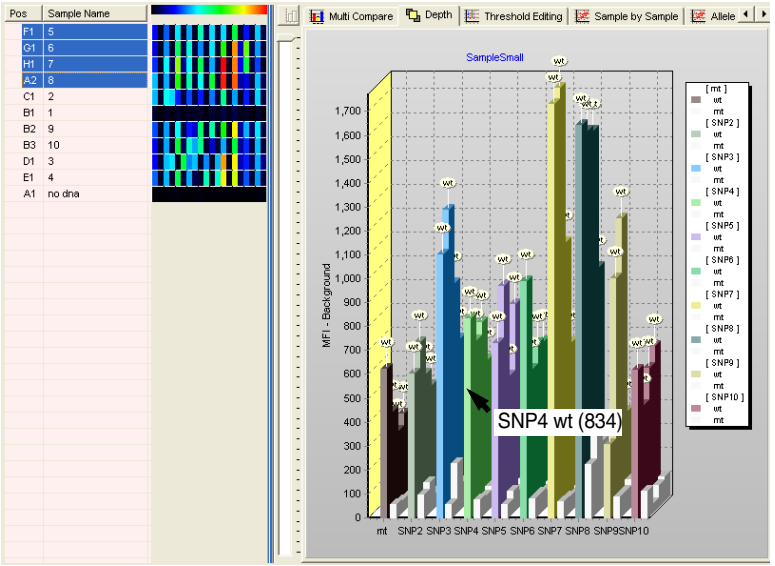


Figure 8.7 Depth graph

Background-adjusted MFI data for samples 5, 6, 7, and 8.

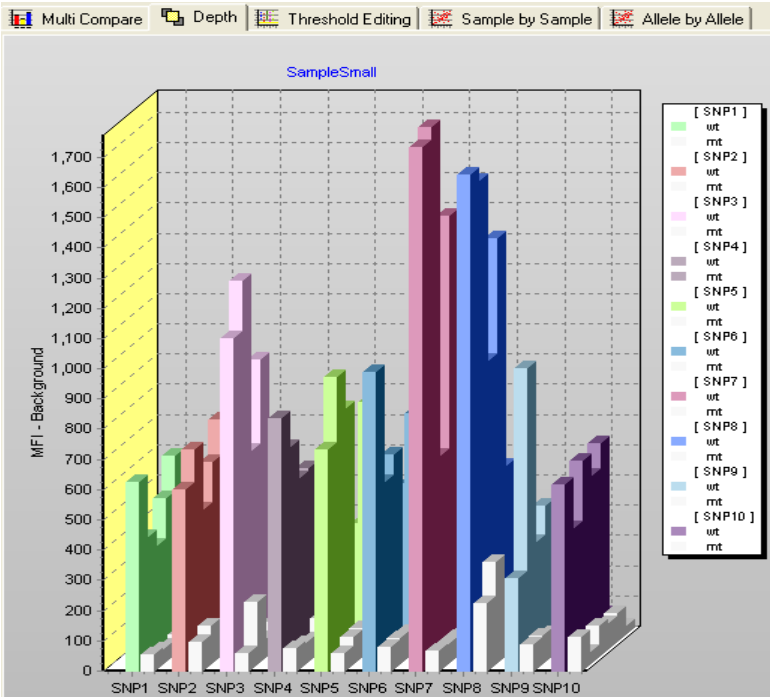


Figure 8.8 Depth graph
Relative intensity data for samples 1-7.

8.5
Multi Compare and Depth Graph Display Options

Several options are available for the Multi Compare or Depth graph display (see Table 8.2 on page 11). You can also modify the graph view in the following ways:

- change the y-axis scale
- increase the graph width or height
- show or hide allele name tags
- reposition the allele name tags
- modify the bottom graph axis labels
- magnify a user-selected area of the graph
- move the graph inside the Project Window

Table 8.2 Graph display options

Default	To...	Click...
3-dimensional (3D) graph	Toggle the view between 3D and 2D view.	
Show name tags for called alleles	Hide or unhide the name tags.	
Display the graph legend	Hide or unhide the legend.	
Display graph bars using solid colors	Toggle the view between a solid or gradient color graph bar.	
Paint only the called alleles	Toggle between paint all alleles or paint only the called alleles	
Display MFI data	Display relative intensity data (RI). Return the display to MFI data.	 
Display the group name of the bead horizontally on the bottom axis of the graph	Display the group names vertically on the bottom axis of the graph.	
Display only the group name of the bead on the bottom axis of the graph	Display the group name and the allele name on the bottom axis of the graph.	
Display all labels on the bottom axis of the graph	Display a subset of the labels so that none overlap on the bottom axis of the graph.	

Changing the Y-Axis Maximum

1. To change the maximum of the y-axis scale, move the slider at the left of the graph (Figure 8.9).

   ⇒ The graph is updated using the new y-axis maximum (the status bar displays the y-axis maximum).

   For example, the Multi Compare graphs in Figure 8.9 plot the same samples using different y-axis maxima.

2. To reset the y-axis maximum to the default, click the button.

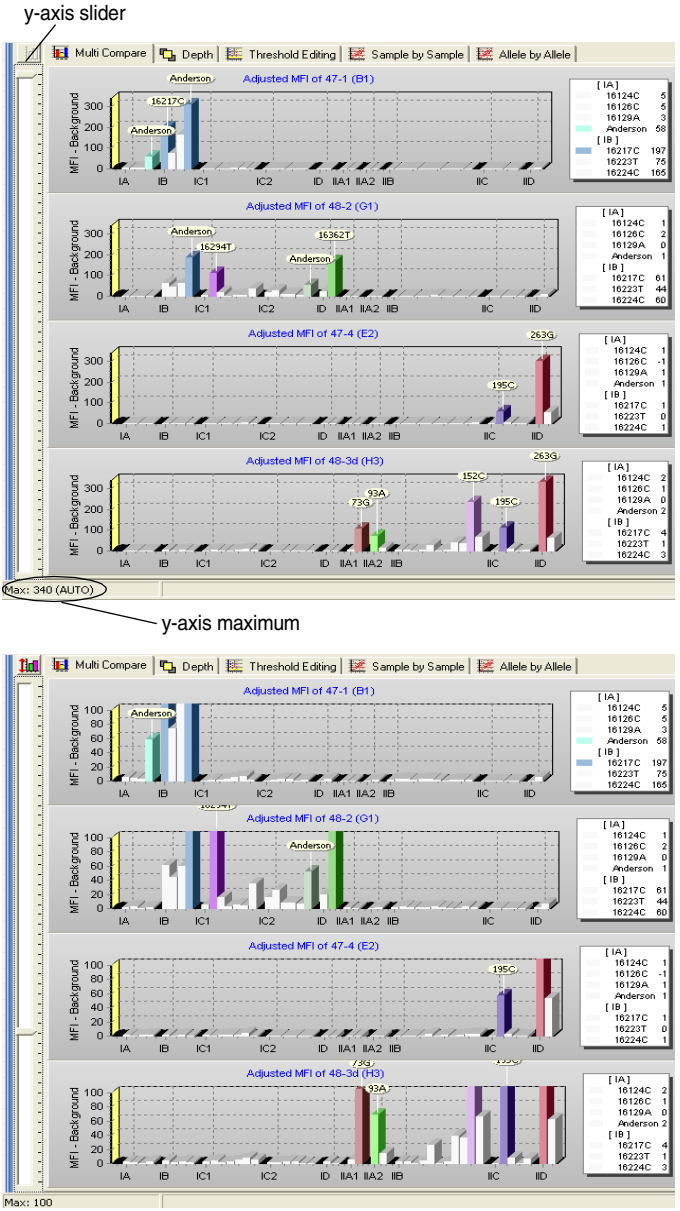


Figure 8.9 Multi Compare graphs
y-axis maximum = 340 (top), y-axis maximum = 100 (bottom).

Adjusting the Graph Width or Height

1. To change the graph width, click the H x1.0 button and select a factor from the drop-down list.
   ⇒ The graph bars and graph width are increased (or decreased) by the selected factor.

   If necessary, use the scrollbar at the bottom of the Project Window to view the Multi Compare graphs.

2. To change the graph height, click the V x1.0 button and select a factor from the drop-down list.
   ⇒ The default graph bars and graph height are increased by the selected factor.

   If necessary, use the scrollbar at the right of the Project Window to view the Multi Compare graphs.

NOTE: If the default graph view does not display the entire graph legend, increase the graph height to view the complete legend.

Repositioning the Allele Name Tags

1. To rotate the position of the allele name tags, move the slider at the top of the Project Window (Figure 8.10).
   ⇒ The name tags are rotated in a counter-clockwise direction.

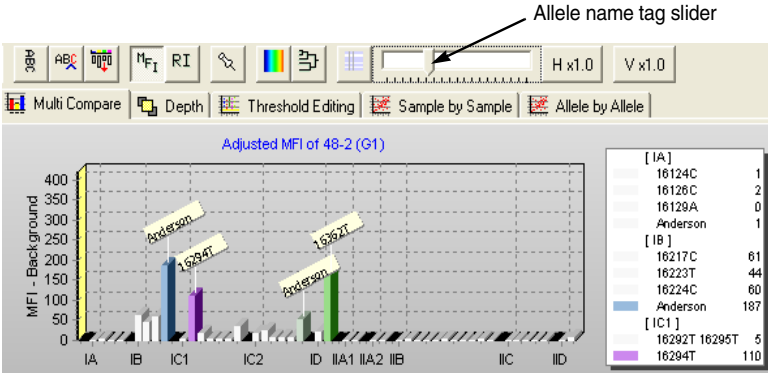


Figure 8.10 Multi Compare graph
Rotated allele name tags.

2. To manually reposition a name tag, click and hold the tag, and move it to a new position.

Magnifying the Graph

You can magnify or zoom in on a user-selected area of the graph.

1. To zoom in on the graph, click and hold the mouse while you draw a rectangle (from upper left to lower right corner) over the area of interest (Figure 8.11).
   ⇒ The Project Window displays the selected graph area (Figure 8.12).

2. To zoom out and return to the original magnification, click and hold the mouse while you draw a rectangle (from right to left) in the graph.
   ⇒ The Project Window displays the graph at the original magnification.

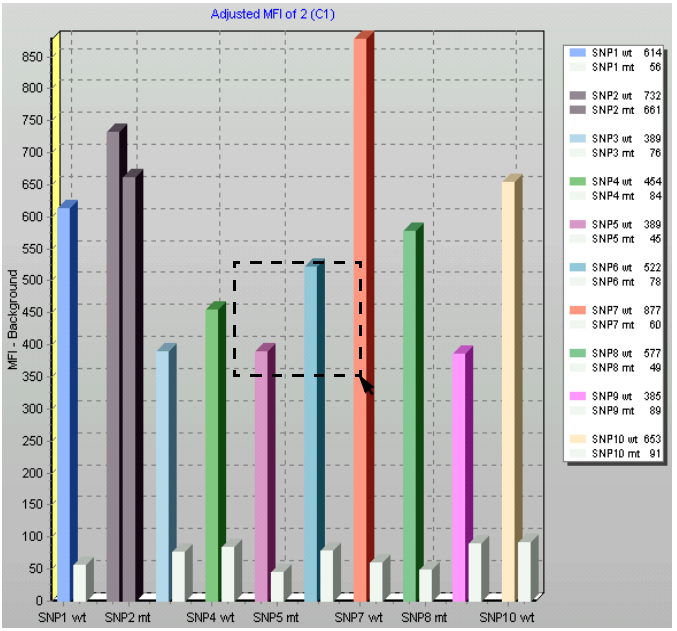


Figure 8.11 Multi Compare graph
Rectangle specifies the area to be magnified.

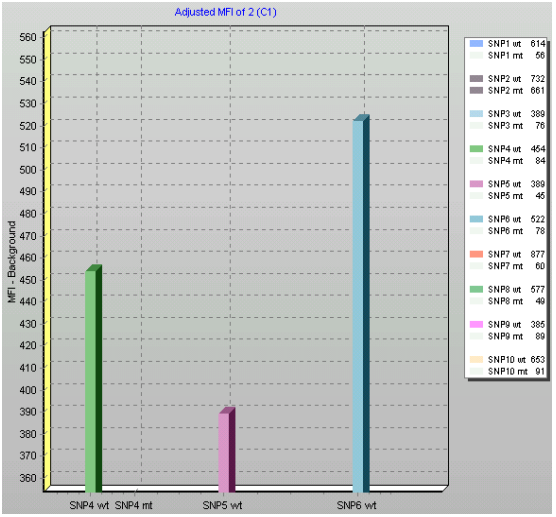


Figure 8.12 Multi Compare graph
User-selected area magnified.

Moving the Graph

You can manually move the graph view in the Project Window.

1. Put the mouse pointer over the graph, then press and hold the right mouse button.
2. To move the graph, move the mouse pointer.

8.6 Threshold Editing

In the Threshold Editing tab, you can:

- view the sample MFI or relative intensity data for a user-selected sample (Figure 8.13 and Figure 8.14).
- view the intensity thresholds for the Relative Intensity Allele Call option set in the Parameter Settings dialog box. (For more information, see *Relative Intensity Allele Call* on page 6.3.)
- change the intensity thresholds

1. To view the intensity data and thresholds, click a sample in the sample list. Click the MFI toolbar button to view MFI data or the RI button to view relative intensity data.

   ⇒ The Threshold Editing tab displays a graph of the intensity data for the selected sample.

   The colored points in the graph represent alleles called in the sample; white points represent alleles that were not called.

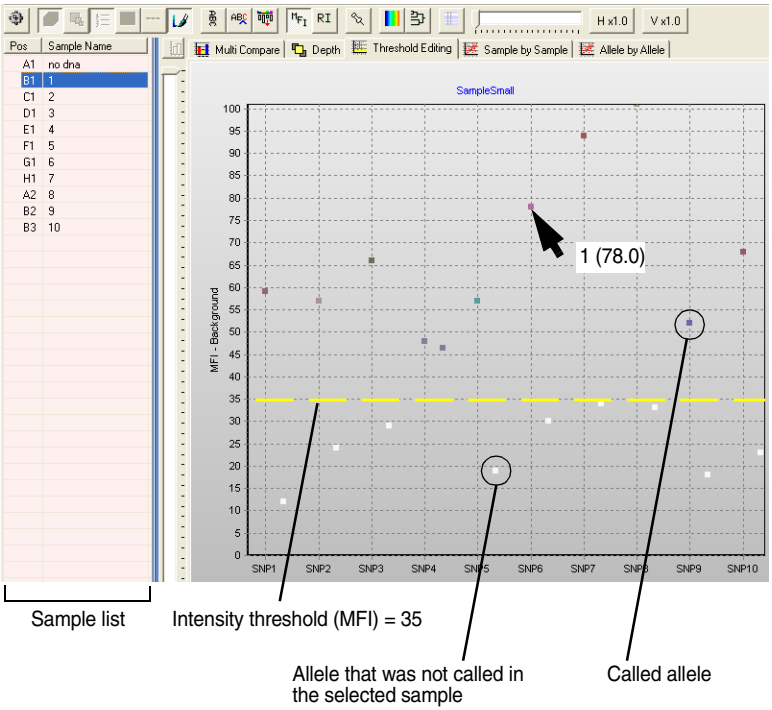


Figure 8.13 Threshold Editing tab
MFI data

2. To view a pop-up tool tip that displays the sample name and intensity data, position the mouse pointer over a graph point.

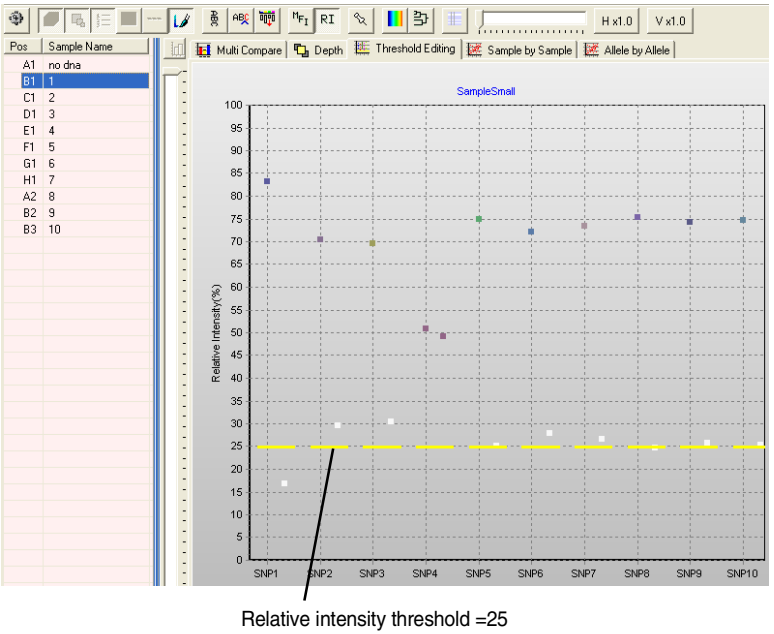


Figure 8.14 Threshold Editing tab
Relative intensity data.

NOTE: The allele is called in the sample if the intensity data exceed the MFI threshold and the relative intensity threshold.

Changing the Intensity Thresholds

You can change the MFI or relative intensity threshold for individual alleles.

1. Position the mouse pointer over the threshold segment that you want to change.
   ⇒ The mouse pointer changes to a ↕.

2. Use the drag-and-drop method to move the threshold to a higher or lower intensity.
   ⇒ The allele calls are updated using the new threshold.The new intensity threshold is displayed in the Parameter Settings dialog

box. (For more information see *Parameter Settings and Options* on page 6.1.)

8.7
Sample by Sample Scatter Graph

The Sample by Sample scatter graph plots the allele (bead set) MFI data for two user-selected samples. The graph displays the correlation coefficient value (R^2) for the two samples and distinguishes between alleles that are called in both samples, only the x-axis sample, only the y-axis sample, or neither sample.

1. Open the Multi Graph view for the results you want to graph and click the Sample by Sample tab.
2. Click the button and click the Sample by Sample tab.
   ⇒ The Multi Graph view is now in the *two sample comparison mode*.

NOTE: To plot a scatter graph, the Multi Graph view must be in the two sample comparison mode.

3. In the Sample Name list (Figure 8.15), right-click one of the samples you want to plot in the scatter graph and select **Sort By Expression** from the shortcut menu that appears.
   ⇒ The selected sample is moved to the top of the sample list and the remaining samples are sorted by similar expression level (MFI data, descending order).
4. In the sample list, click the second (y-axis) sample for the scatter plot.
   ⇒ The Sample by Sample scatter plot is displayed (Figure 8.15).

   The graph points are identified by color:

Graph Point Color	Represents an allele that is...
White	Not called in either sample.
Red (default)	Called only in the y-axis sample.
Blue (default)	Called only in the x-axis sample.
Black	Called in both samples.

NOTE: You can change the red and blue default colors for alleles in the Sample by Sample scatter graph in the Application Options dialog box. (See *Changing the Gradient Background Colors* on page A.3.)

5. To view an allele name tag, put the mouse pointer over a graph point.
   ⇒ A pop-up tool tip displays the allele name.

6. To display all of the allele name tags, click the graph.
   ⇒ All of the allele name tags are displayed in the graph (Figure 8.16).

7. To plot a a new scatter graph that includes the sample at the top of the sample list, click another sample.
   ⇒ The Sample by Sample scatter graph for the two samples is displayed.

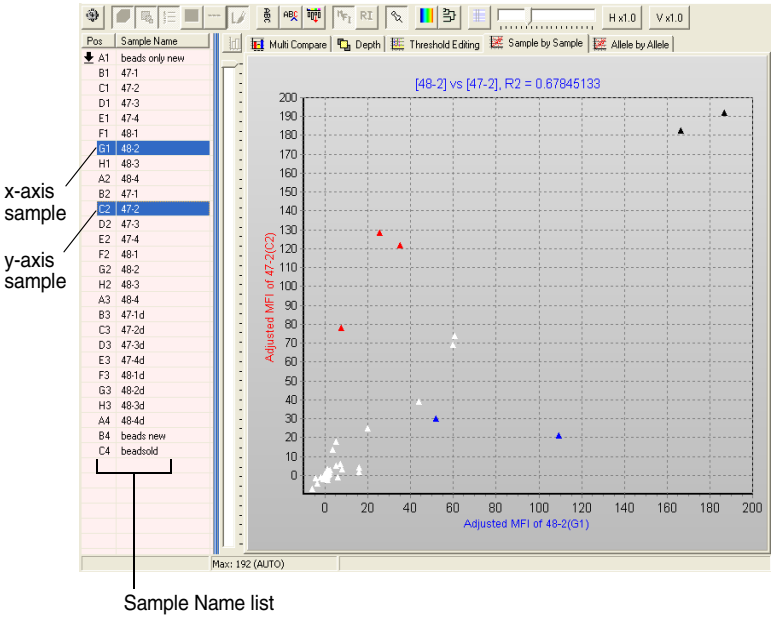


Figure 8.15 Sample by Sample scatter plot

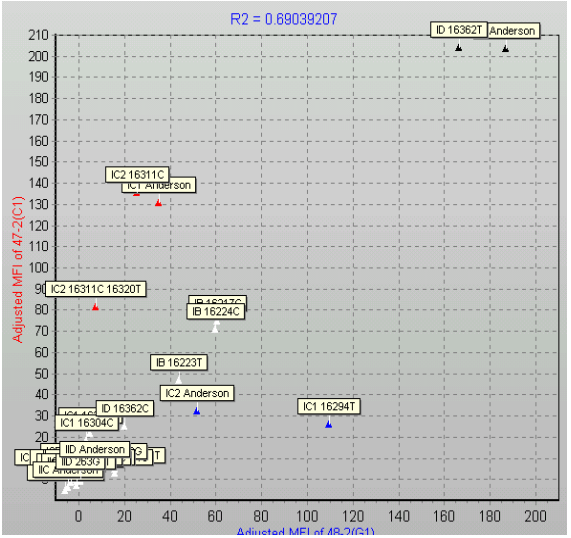


Figure 8.16 Sample by Sample scatter plot
Allele labels displayed.

8.8
Allele by Allele Scatter Graph

The Allele by Allele scatter graph plots the MFI data for two user-selected alleles from all samples in the active results. The scatter graph distinguishes between samples in which both alleles are called, neither allele is called, only the x-axis allele is called, or only the y-axis allele is called.

1. Open the Multi Graph view for the results you want to graph and click the Allele by Allele tab.
2. In the allele list (Figure 8.17), press and hold the **Shift** key while you click the two alleles for the scatter graph.
   ⇒ The Allele by Allele scatter graph for the user-selected alleles is displayed (Figure 8.17).

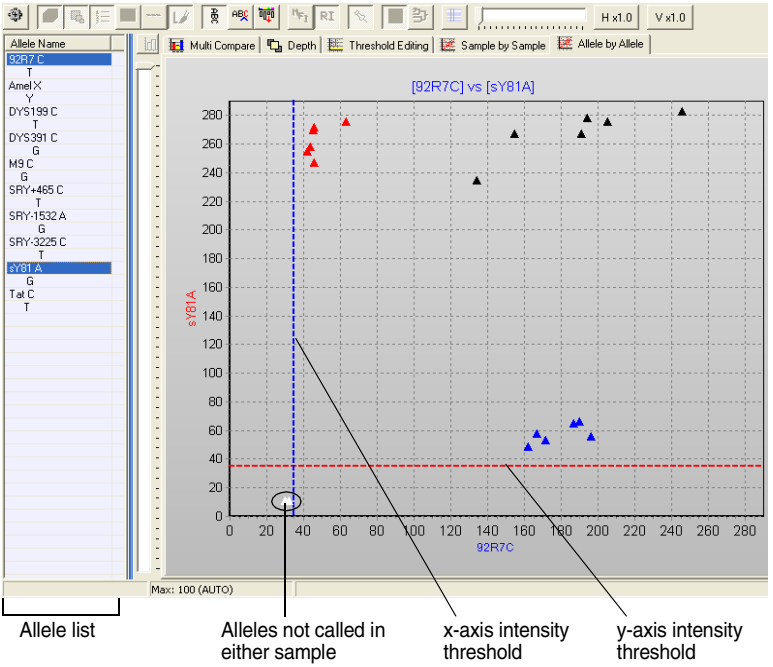


Figure 8.17 Allele by Allele scatter graph

The graph points are identified by color:

Graph Point Color	Represents a sample in which...
White	Neither allele is called.
Red (default)	Only the x-axis allele is called.
Blue (default)	Only the y-axis allele is called.
Black	Both alleles are called.

NOTE: You can change the red and blue default colors in the Allele by Allele scatter graph (for intensity thresholds and graph points) in the Application Options dialog box. (See *Changing the Gradient Background Colors* on page A.3.)

8.9
Copying a Graph

To copy a graph, right-click the graph and select one of the following from the shortcut menu that appears:

Copy as a Bitmap	Copies the graph in bitmap format (.bmp) to the system clipboard.
Copy as Windows MetaFormat	Copies the graph in Windows metaformat (.emf) to the system clipboard.
Copy All Charts as a Bitmap	Copies all graphs in bitmap format (.bmp) to the system clipboard.

8.10
Printing a Graph

To print a graph, right-click the graph and select **Print Chart** from the shortcut menu that appears.

8.11
Adding Graphs to a Report

1. Right-click the graph and select **Add to Report** in the shortcut menu that appears.
2. If you want to add all graphs that have been plotted to a report, right-click the Multi Compare graph and select **Add All Charts to Report** in the shortcut menu that appears.

CHAPTER 9

CLUSTER ANALYSIS

This chapter explains how to apply a cluster analysis to the samples and display a sample dendrogram. The MasterPlex™ GT software can apply cluster analysis to sample genotype or expression data using the following methods:

- Nearest Neighbor Minimum
- Farthest Neighbor Maximum
- Median
- Centroid
- Between Group Link
- Ward's
- Flexible

9.1 Displaying a Dendrogram

The Clustering Tool is available in the Typing table or Multi Graph view (except for the Allele by Allele tab).

1. To display the Multi Graph view of:
   - the active results (.csv or .gtp), click the  button
   - a particular project, click **Multi Graph** under the project of interest in the Project Manager (Figure 9.1)
2. To display the Typing table for:
   - the active results, click the  button
   - a particular project, click **Typing Table** under the project of interest in the Project Manager (Figure 9.1)

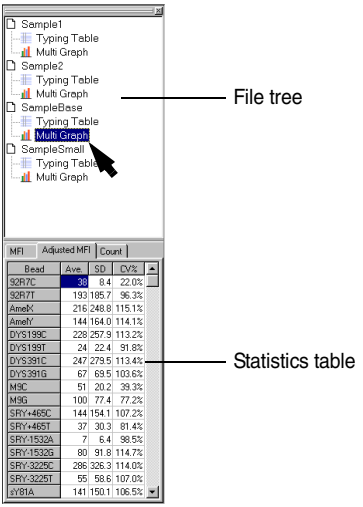


Figure 9.1 Project Manager

3. Click the **Show Dendrogram** toolbar button .

   ⇒ The Clustering Tool window (Figure 9.2) and the dendrogram (Figure 9.3 and Figure 9.4) are displayed.

   The Wards method and cluster by genotype are the defaults. The **Genotype** option clusters samples according to the genotype called for the alleles. The **Expression** option clusters samples according to the MFI data for the alleles.

NOTE: The **Show Dendrogram** button is not available in the Allele by Allele tab.

Figure 9.2 Clustering Tool window

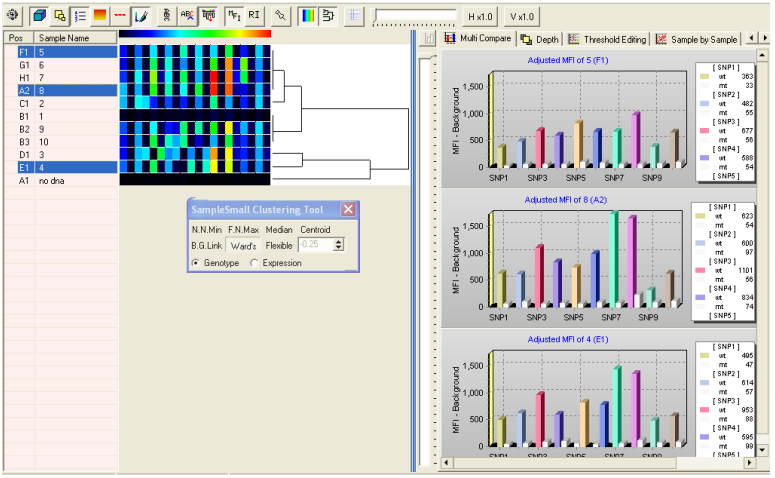


Figure 9.3 Dendrogram in the MultiGraph view

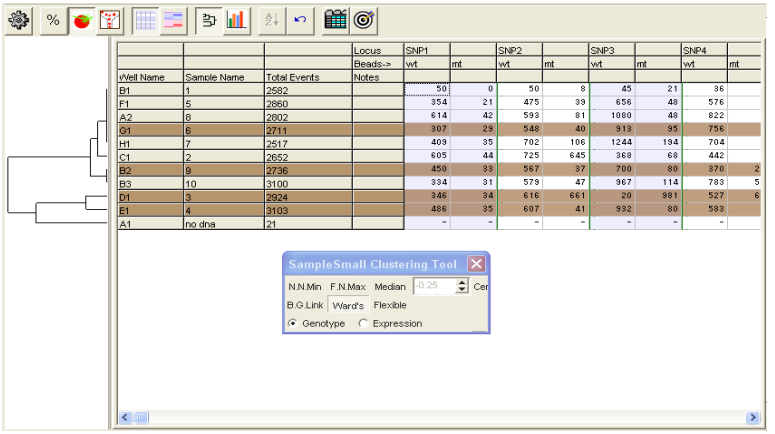


Figure 9.4 Dendrogram in the Typing table

4. To select a different clustering tool, click the tool name in the Clustering Tool window.
   ⇒ The dendrogram is updated.

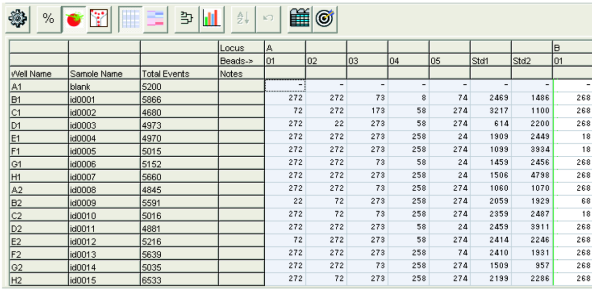
5. To close the dendrogram, click the **Close** button in the Clustering Tool window.

CHAPTER 10 GENOTYPING USING A LOOKUP TABLE

The MasterPlex™ GT software can use a lookup table (.gtt) to call genotypes. As an example, this chapter explains the steps to HLA typing using a lookup table, and how to import or setup, and manage lookup tables.

Figure 10.1 and Figure 10.2 provide an overview of the steps to perform HLA typing in MasterPlex GT using a lookup table.

1. Open Luminex results (.csv).



2. Import or create a HLA lookup table (.gtt). To import a table, double-click the .gtt or drag it to the MasterPlex GT application window.

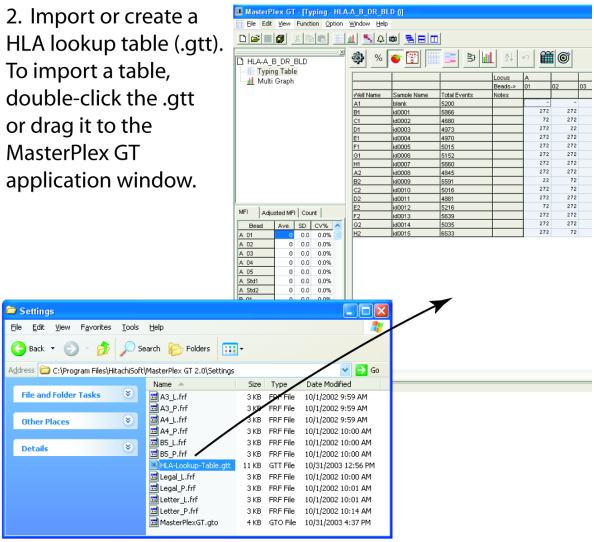
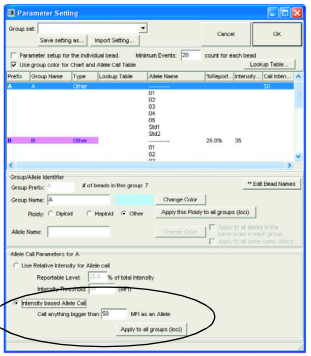
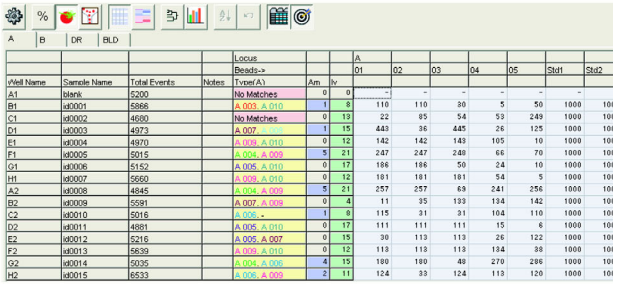


Figure 10.1 Steps to type samples using a HLA lookup table
Steps continued in Figure 10.2.

3. Set thresholds (or select a group set).
Choose Intensity-based allele call.
Call anything bigger than 50 MFI an allele.
Click **Apply to all groups**.



4. View the Typing table.
Click a tab to select a locus or the bloodtype.



				Locus			A						
				Beads->			01	02	03	04	05	Std1	Std2
Well Name	Sample Name	Total Events	Notes	Type(A)	Am	lv							
A1	blank	5200		No Matches	0	0	-	-	-	-	-	-	-
B1	id0001	5886		A 003 , A 010	1	8	110	110	30	5	50	1000	10
C1	id0002	4680		No Matches	0	19	22	85	54	53	249	1000	10
D1	id0003	4973		A 007, -	1	15	443	36	445	26	125	1000	10
E1	id0004	4970		A 008, A 010	0	12	142	142	143	105	10	1000	10
F1	id0005	5015		A 004, A 009	5	21	247	247	248	66	70	1000	10
G1	id0006	5152		A 005, A 010	0	17	186	186	50	24	10	1000	10
H1	id0007	5660		A 008, A 010	0	12	181	181	181	54	5	1000	10
A2	id0008	4845		A 004, A 009	5	21	257	257	69	241	256	1000	10
B2	id0009	5591		A 007, A 009	0	4	11	35	133	134	142	1000	10
C2	id0010	5016		A 005, -	1	8	115	31	31	104	110	1000	10
D2	id0011	4881		A 005, A 010	0	17	111	111	111	15	6	1000	10
E2	id0012	5216		A 005, A 007	0	15	30	113	113	26	122	1000	10
F2	id0013	5639		A 009, A 010	0	12	113	113	113	134	38	1000	10
G2	id0014	5035		A 004, A 006	4	15	180	180	48	270	286	1000	10
H2	id0015	6533		A 006, A 009	2	11	124	33	124	113	120	1000	10

Click:  to view by locus.

 to show the type.

 to display the Typing table with gradient background.

an Am number to show ambiguity candidates.

an Lv number to show inversion candidates.

Figure 10.2 Steps to type samples using a HLA lookup table

10.1
Importing a Lookup Table

The import process is carried out only once for each lookup table (.gtt). To import a lookup table:

1. Open the MasterPlex™ GT software.
2. In Windows Explorer, navigate to the lookup table (.gtt).

NOTE: Lookup tables for import should be located on the desktop or a folder other than the Settings folder. The software copies the .gtt to the Settings folder.

3. Double-click the file or drag the file to the MasterPlex GT application window (Figure 10.3).
   ⇒ The lookup table is installed (copied to the Settings folder) and the file name is added to the Lookup Table Selection window (Figure 10.5).

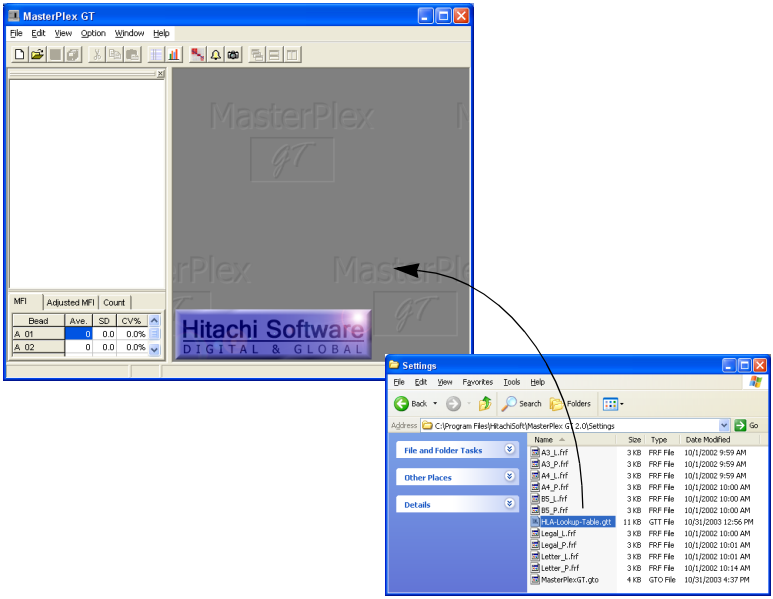


Figure 10.3 Importing a lookup table
To import a lookup table, drag the .gtt file to the MasterPlex GT application window or double-click the file.

4. To confirm the lookup table import:
   a. Open the Luminex® results file (.csv) or MasterPlex™ GT project (.gtp) of interest.
   b. Click the **Parameter Setting** button .
      ⇒ The Parameter Setting dialog box appears (Figure 10.4).

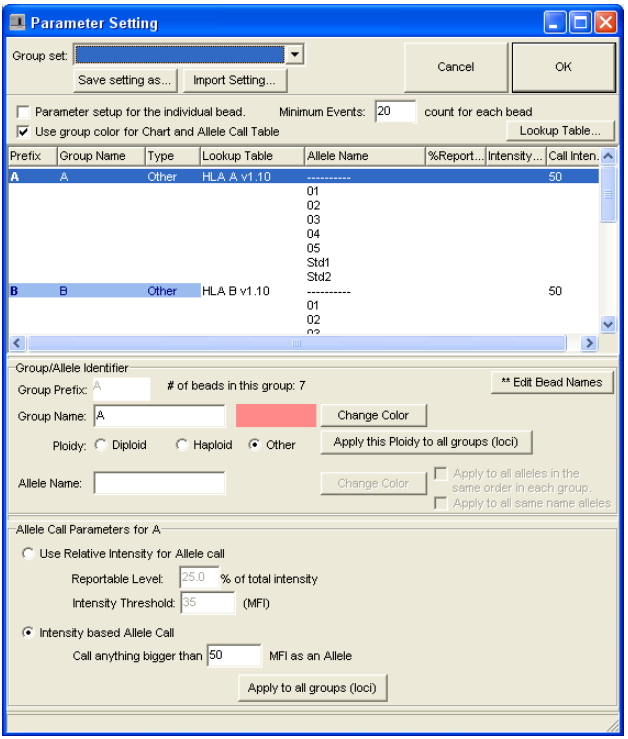


Figure 10.4 Parameter Setting dialog box

   c. Click **Lookup Table**.
      ⇒ The Lookup Table Selection window appears (Figure 10.8).
   d. Confirm that the Lookup table Selection window includes the name of the table (Entry Name) that you imported.

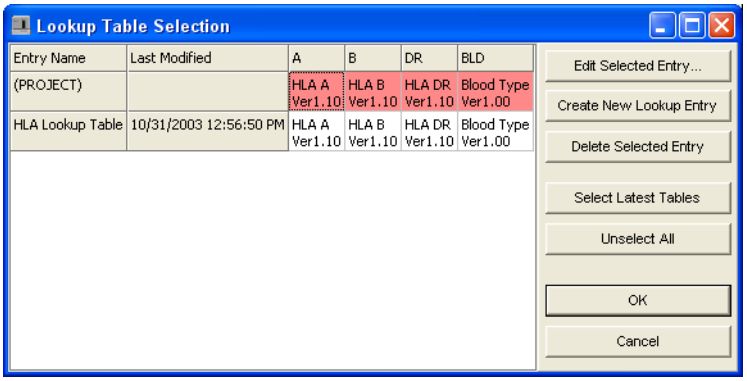


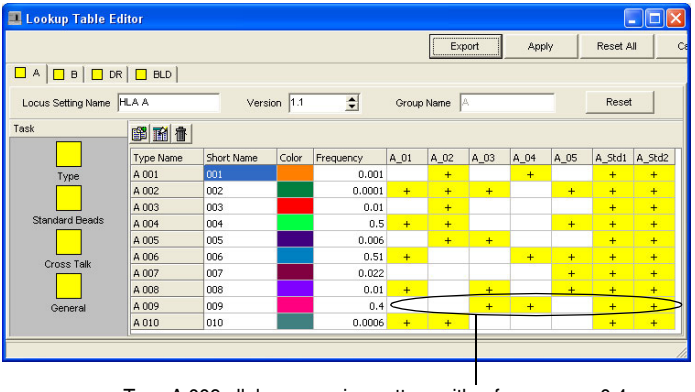
Figure 10.5 Lookup Table Selection window
This window shows the lookup tables installed in the MasterPlex™ GT software.

Component in the Lookup Table Selection Window	Function
Edit Selected Entry	Opens the Lookup Table Editor for the selected table.
Create New Lookup Entry	Opens the New Lookup Table Entry dialog box so that you can name a new lookup table.
Delete Selected Entry	Deletes the selected lookup table.
Select Latest Table	Selects the latest lookup table version for all loci.
Unselect All	Clears the selection from the Lookup Table Selection window.
OK	Accepts the lookup table selection and returns to the Parameter Setting dialog box.
Cancel	Selects the default lookup table and returns to the parameter Setting dialog box.

10.2
Creating a Lookup Table

The Lookup Table Editor (Figure 10.6) enables you to specify the table components, including the:

- Table name
- Type (genotype) name for the A, B, and DR loci and the blood type
- Type frequency
- Standards and allele expression patterns that define the type
- Display color for the type name in the MasterPlex GT Typing table



Type A 009 allele expression pattern with a frequency = 0.4.

Figure 10.6 Lookup Table Editor, Type task view

1. Open the Luminex® results file (.csv) or project (.gtp) of interest.
2. Click the **Parameter Setting** button .
   ⇒ The Parameter Setting dialog box appears (Figure 10.7).

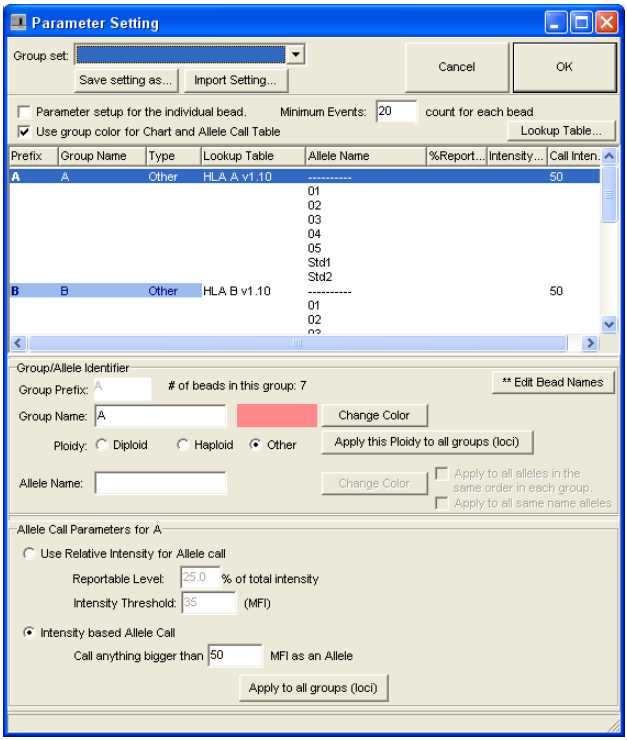


Figure 10.7 Parameter Setting dialog box

3. Click **Lookup Table.**
   ⇒ The Lookup Table Selection window appears (Figure 10.8).

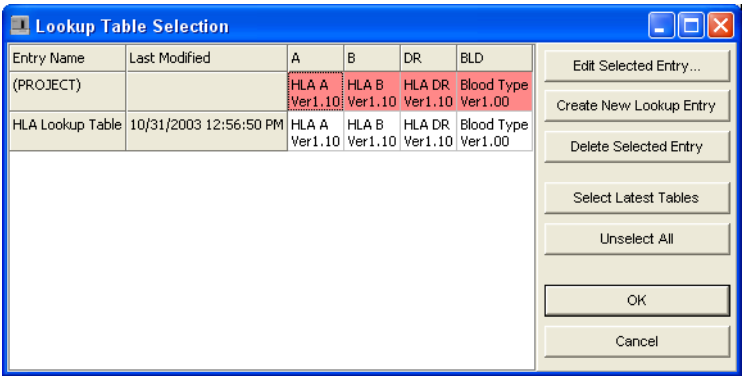


Figure 10.8 Lookup Table Selection window
This window shows the available lookup tables and the table applied to the current project. In this window you can create a new lookup table or edit a table.

4. In the Lookup Table Selection window (Figure 10.8), click **Create New Lookup Entry**.
   ⇒ The New Lookup Table Entry dialog box appears (Figure 10.9).

Figure 10.9 New Lookup Table Entry dialog box

5. Enter a name for the new lookup table and click **OK**.
   ⇒ The Lookup Table Editor appears (Figure 10.10).

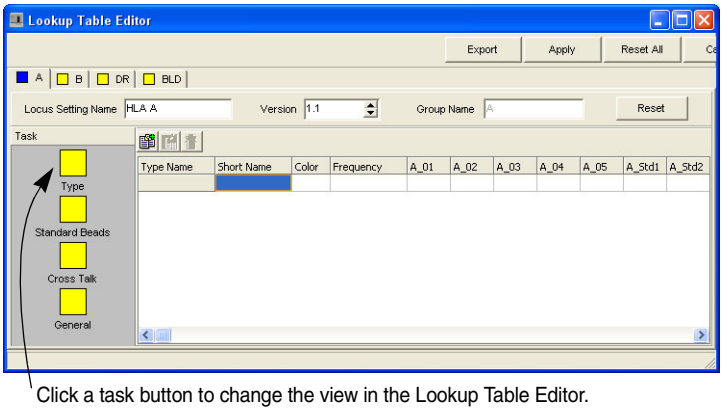


Figure 10.10 Lookup Table Editor, Type view

Lookup Table Editor Button	Function
Export	Opens the Export wizard.
Apply	Applies the changes to the selected lookup table and close the Lookup Table Editor.
Reset All	Removes all changes from every locus.
Reset	Removes the changes from the currently selected locus.
Cancel	Closes the Lookup Table Editor without applying any changes.

Defining a Type

The Type view is the default in the Lookup Table Editor (Figure 10.10). In this view, you can specify or edit the allele expression pattern that defines a type.

1. In the Lookup table Editor, select a locus tab (Figure 10.11).
2. Enter a Locus Setting Name.

   For example, in Figure 10.10, the A tab is selected, and HLA A was entered for the locus setting name. The Lookup table Selection window will display this name.

3. Enter a version number for the selected locus or blood type (Figure 10.11).

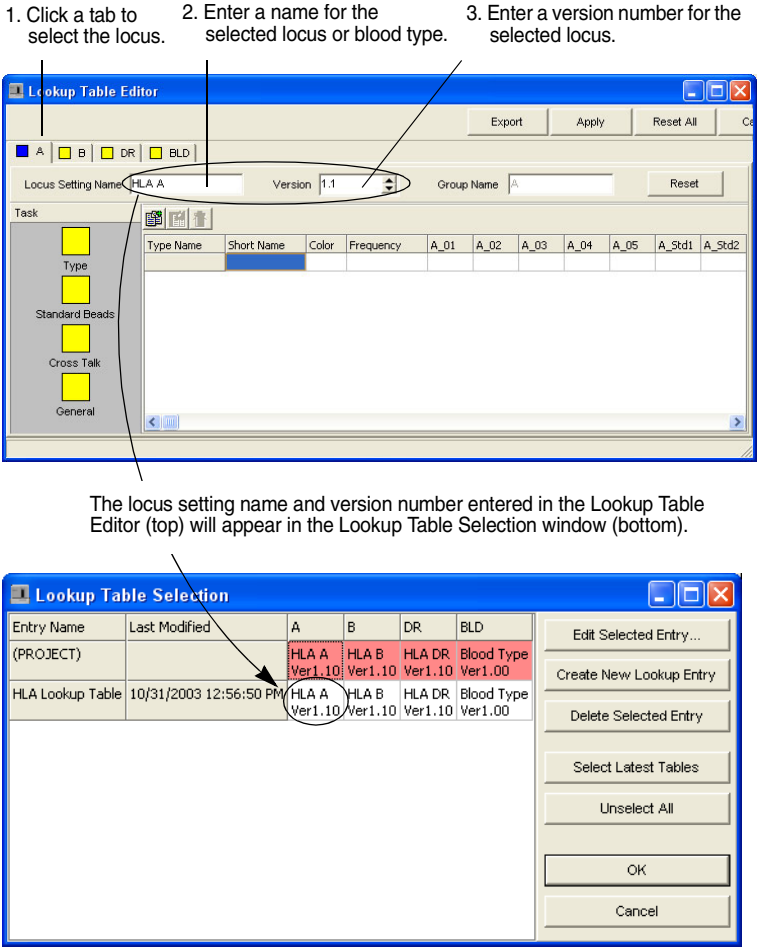


Figure 10.11 Lookup Table Editor, Type view (top); Lookup Table Selection window (bottom).

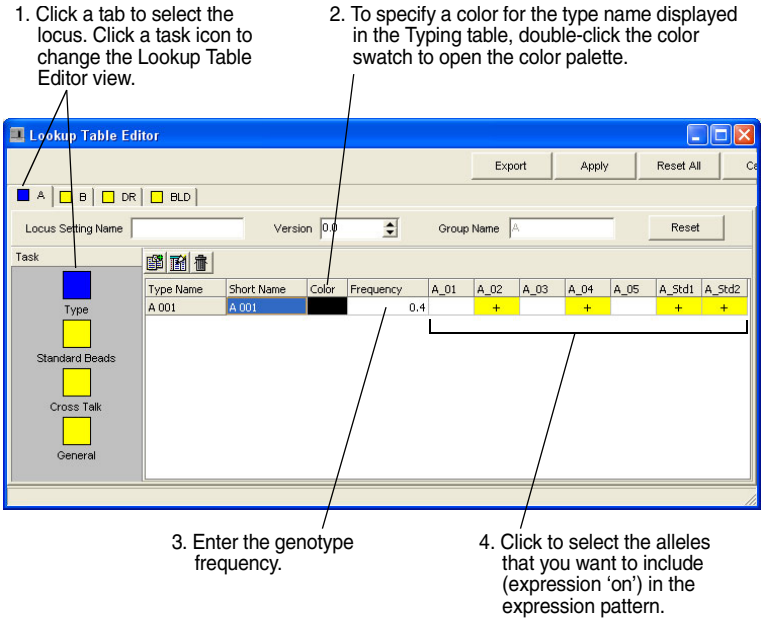


Figure 10.12 Lookup Table Editor, Type view

The Type icon and locus tab icon change from yellow to blue ( ) to indicate the type has been edited.

Lookup Table Editor Component	Description
Type Name	Genotype name.
Short Name	Not used in MasterPlex GT 2.0.
Frequency	Genotype expression frequency. If a type has ambiguity candidates, this value determines the order of a call and its ambiguity candidates in the Typing table. Frequency must be > 0.

4. Click the **Add New** button . Alternatively, right-click the Lookup Table Editor and select **Add New** from the shortcut menu that appears.

   ⇒ The New Type Name Entry dialog box appears (Figure 10.13).

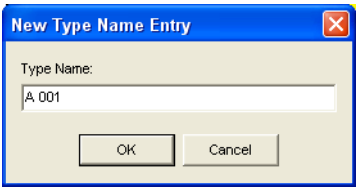


Figure 10.13 New Type Name Entry dialog box

5. Enter a type (genotype) name for the selected locus or blood type and click **OK**.
   ⇒ The Lookup Table Editor displays the new type (genotype) name (Figure 10.12).

NOTE: The short name is not used in MasterPlex GT 2.0.

6. To edit a type name, select the type name and click the **Edit Type Name** button. Alternatively, right-click the Lookup Table Editor, and select **Edit Type Name** from the shortcut menu that appears.
   ⇒ The Edit Type Name Entry dialog box appears (Figure 10.14).

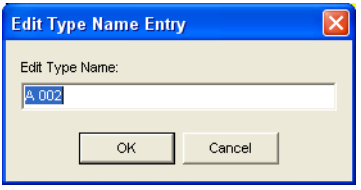


Figure 10.14 Edit Type Name Entry dialog box

7. To delete a type name, select the type name and click the **Delete** button. Alternatively, right-click the Lookup Table Editor, and select **Delete** from the shortcut menu that appears. Click **Yes** in the confirmation message box that appears.
8. To specify the display color of the type name in the Typing table:
   a. Double-click the color swatch.
   ⇒ The color palette appears (Figure 10.15).

Figure 10.15 Color palette

b. To select a predefined color, click one of the basic colors.

c. To define a custom color, click **Define Custom Colors**.

⇒ The color palette shows the custom color options (Figure 10.16).

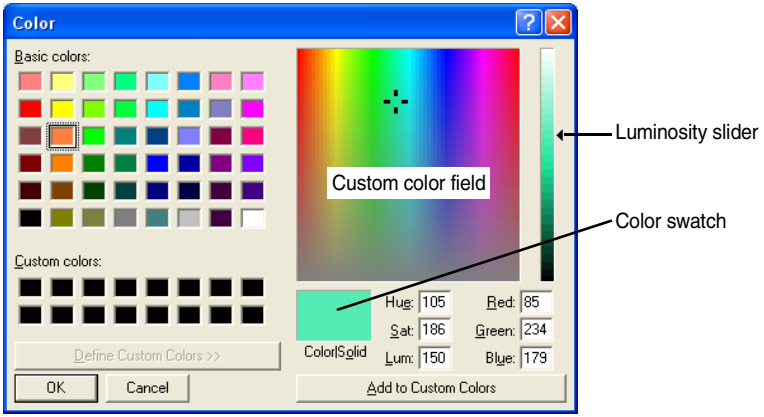


Figure 10.16 Color palette
Custom color options.

d. Use the click-and-drag operation to move the cross hairs in the custom color field. Adjust the color brightness using the luminosity slider.

⇒ The Color swatch shows the color selection.

e. When you are finished defining the color, click **Add to Custom Colors** to apply the color, and click **OK**.

9. Enter the genotype frequency.
10. Click the alleles and standards that you want to include in the genotype expression pattern.
11. To define another allele expression pattern for the selected locus, follow step 4 to step 10.
12. To define the allele expression patterns for another locus or the blood type, follow step 1 to step 11.
13. To copy the
14. Click **Apply** when you finish defining the genotype expression patterns.

Setting Standards

In the Standard Beads view of the Lookup Table Editor (Figure 10.17), you can:

- Associate alleles or a blood group with a standard.
- Choose normalization for a standard and the associated allele MFI data.
- Set a base MFI threshold for a standard MFI. When the normalization option is chosen, the data are only normalized if the standard MFI > base MFI.
- Set a cutoff MFI value. If the standard MFI < cutoff MFI, the data are not normalized.

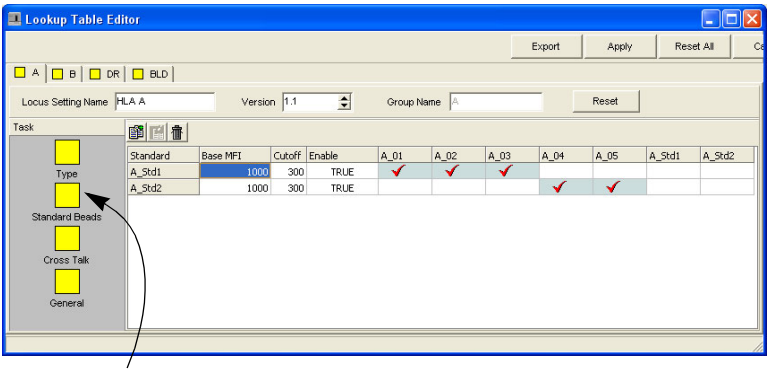


Figure 10.17 Lookup Table Editor, Standard Beads view
This view shows the standards associated with the alleles of the selected locus.

1. In the Lookup Table Editor, click a tab to select a locus.
2. Click the **Standard Beads** button .
   ⇒ The Standard Beads view for the selected locus or blood type appears (Figure 10.18).

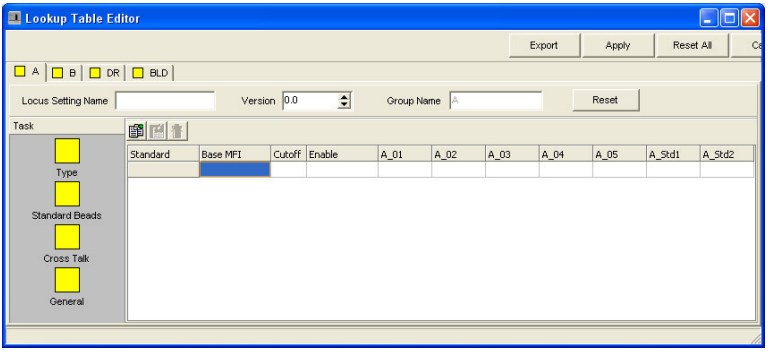


Figure 10.18 Lookup Table Editor, Standard Beads view

3. Click the **Add New** button . Alternatively, right-click the Lookup Table Editor and select **Add New** from the shortcut menu that appears.
   ⇒ The New Standard Bead Name Entry dialog box appears (Figure 10.19).

Figure 10.19 New Standard Bead Name Entry dialog box

4. Click the drop-down arrow and make a selection from the drop-down list, and click **OK**.
   ⇒ The standard is added to the Lookup Table Editor (Figure 10.20).

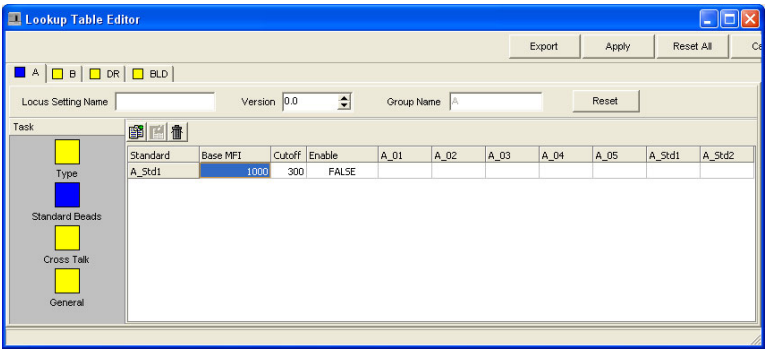


Figure 10.20 Lookup Table Editor, Standard Beads view
Confirm the default base MFI and cutoff values or enter new values.

5. Confirm the default base MFI value or enter a new value.

   The software uses the base MFI value to normalize the MFI values of the alleles that are associated with the standard. To normalize the data, the software sets the standard MFI equal to the Base MFI and computes:

   Normalized Allele MFI = Allele MFI x (Base MFI/Standard MFI)

NOTE: The cutoff value must be less than the base MFI value.

6. Confirm the default cutoff value or enter a new value.

   If the standard MFI is less than the cutoff value, the software does not normalize the standard or allele MFI data.

NOTE: The cutoff value must be less than the base MFI and greater than one.

7. To apply normalization to the selected standard at the associated allele MFI data, click the entry in the Enable column and select **TRUE** from the drop-down menu that appears (Figure 10.21).

   If you do not want to normalize the data, select **FALSE**.

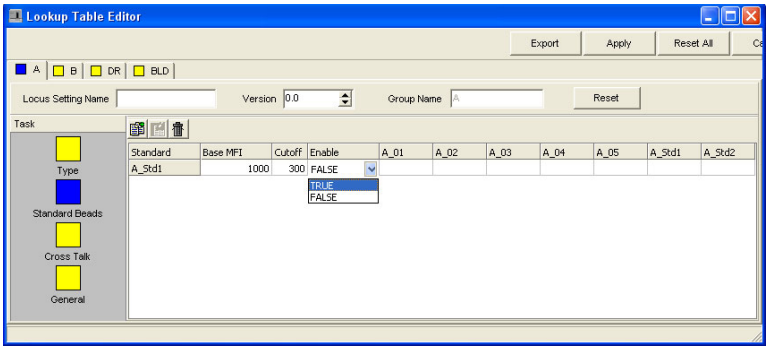


Figure 10.21 Lookup Table Editor, Standard Beads view
Select TRUE from the drop-down list to normalize the standard and associated allele MFI data. If you do not want to normalize the data, select FALSE.

8. To associate alleles with the standard, click the allele columns of interest (Figure 10.22).

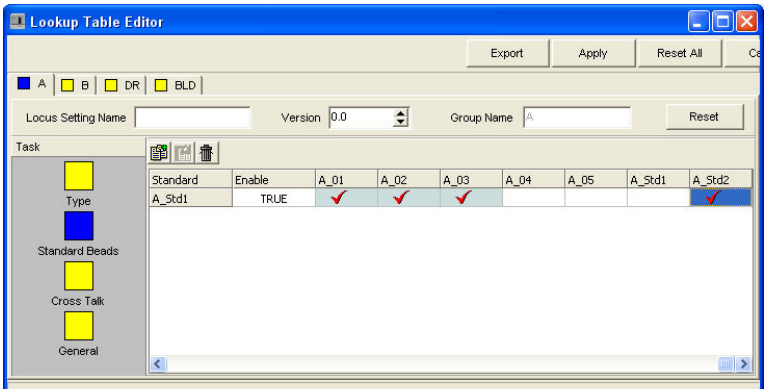


Figure 10.22 Lookup Table Editor, Standard Beads view
Click the alleles that you want to associate with a standard.

9. To reset all entries to the default value, click **Reset**.
10. To specify another standard for the same locus, follow step 3 to step 8.
11. To delete a standard, click the standard row and click the **Delete** button . Alternatively, right-click the Lookup Table Editor and select **Delete** from the shortcut menu that appears. Click **Yes** in the delete confirmation message that appears.
12. To specify standards at another locus, follow step 1 to step 10.

Specifying Cross-talk

In the Cross-talk view of the Lookup Table Editor (Figure 10.17), you can specify the per cent cross hybridization between two bead sets. The software uses this information to compute MFI values that are corrected for cross hybridization.

For example, if bead sets A, B and C exhibit cross hybridization as follows:

10% of allele B binds to bead A

5% of allele C binds to bead A

then, the software computes:

$$\text{Corrected MFI}_A = \text{Original MFI}_A - (0.1*\text{MFI}_B) - (0.05*\text{MFI}_C)$$

1. In the Lookup Table Editor, click a tab to select a locus.
2. Click the **Cross-talk** button .
   ⇒ The Cross-talk view for the selected locus or blood type appears (Figure 10.23).

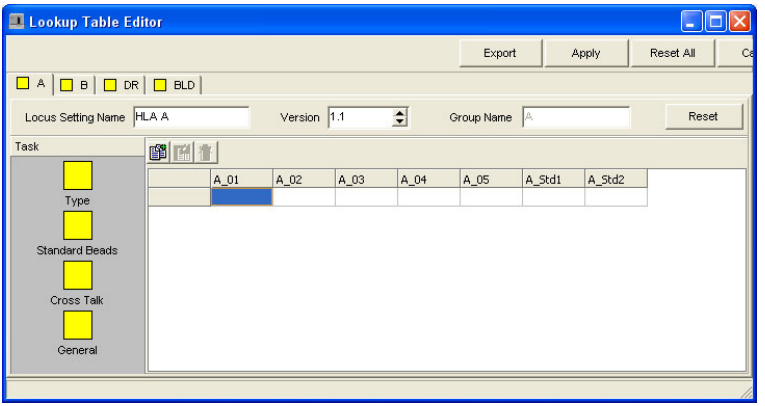


Figure 10.23 Lookup Table Editor, Cross-talk view
In this view, you can specify the per cent cross hybridization between two bead sets.

3. Click the **Add New** button . Alternatively, right-click the Lookup Table Editor and select **Add New** from the shortcut menu that appears.
   ⇒ The New Standard Bead Name Entry dialog box appears (Figure 10.19).

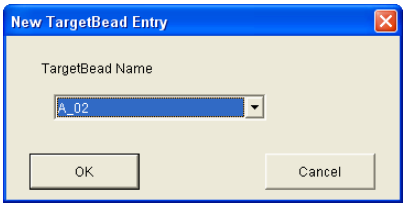


Figure 10.24 New Target Bead Entry dialog box

4. Click the drop-down arrow and make a selection from the drop-down list, and click **OK**.
   The MFI of the selected allele will be corrected for cross hybridization.

5. The bead set is added to the Lookup Table Editor (Figure 10.25).

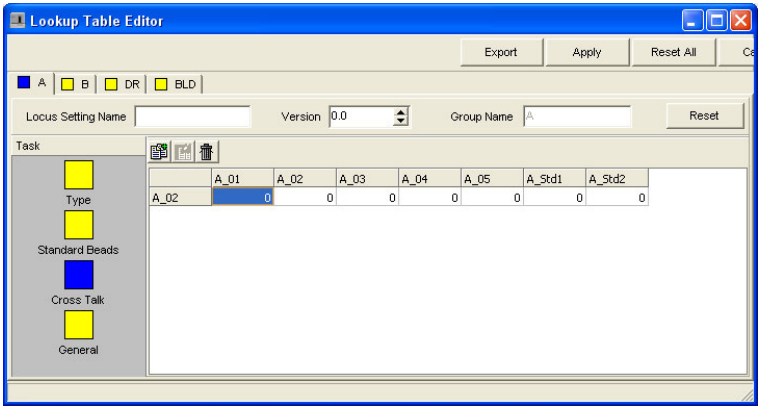


Figure 10.25 Lookup Table Editor, Cross-talk view

6. Enter the per cent cross hybridization data for the allele pairs.

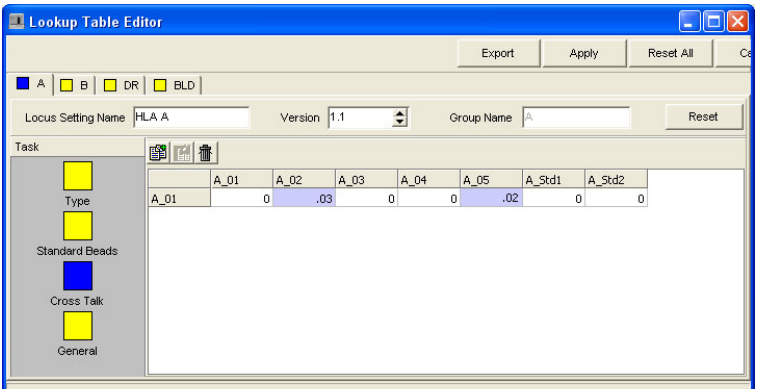


Figure 10.26 Lookup Table Editor, Cross-talk view

In Figure 10.26, the software computes $MFI_{A\,01}$ corrected for cross hybridization as follows:

Corrected $MFI_{A\,01}$ = Original $MFI_{A\,01}$ - (0.03*$MFI_{A\,02}$) - (0.02*$MFI_{A\,05}$)

7. To delete an allele from the cross-talk table, click the row that you want to delete, and click the **Delete** button. Alternatively, right-click the Lookup Table Editor and select **Delete** from the shortcut menu that appears. Click **Yes** in the delete confirmation message that appears.
8. To reset all cross-talk entries to zero (default), click **Reset**.
9. To enter cross hybridization values for another allele, follow step 3 to step 6.
10. To enter cross hybridization values for the alleles at another locus, follow step 1 to step 9.

Setting General Parameters

In the General view of the Lookup Table Editor (Figure 10.17), you can specify:

- The allele ploidy
- The text that the Typing table displays when a sample has no matching genotype
- Whether to display standard data in the Typing table

1. In the Lookup Table Editor, click a tab to select a locus.
2. Click the **General** button.

⇒ The General view for the selected locus or blood type appears (Figure 10.23).

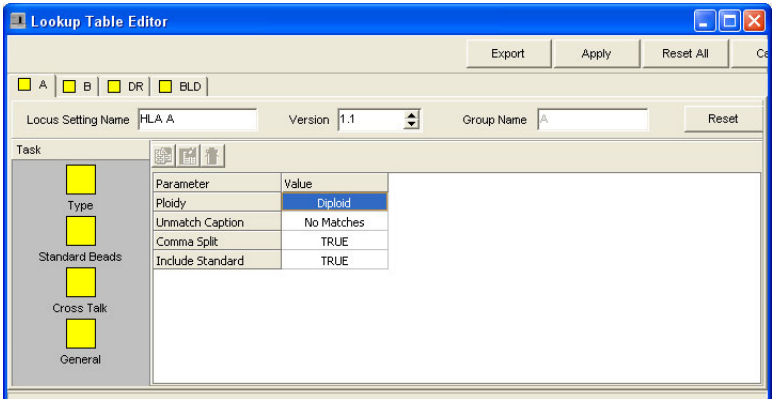


Figure 10.27 Lookup Table Editor, General view

Parameter	Description
Ploidy	Choose diploid, haploid, or other.
Unmatch Caption	The default unmatch caption is **No Matches**. The Typing table displays **No Matches** when there is no genotype match for a sample. The caption is user-editable.
Comma Split	Describes how the Typing table displays a genotype. The comma split display (for example, A 002, A 010) is the only option available at this time.
Include Standard	TRUE = include standard data in the Typing table. FALSE = do not include standard data in the Typing table.

3. To select a ploidy option, click the ploidy value and make a selection from the drop-down list that appears (diploid, haploid, or other).
4. To edit the unmatch caption, double-click the value and enter a new value.

10.3
Managing Lookup Tables

You can edit, export, copy, or delete a lookup table.

Editing a Lookup Table

1. Open the Luminex® results (.csv) or project (.gtp) of interest.
2. Click the **Parameter Setting** button .
   ⇒ The Parameter Setting dialog box appears (Figure 10.28).

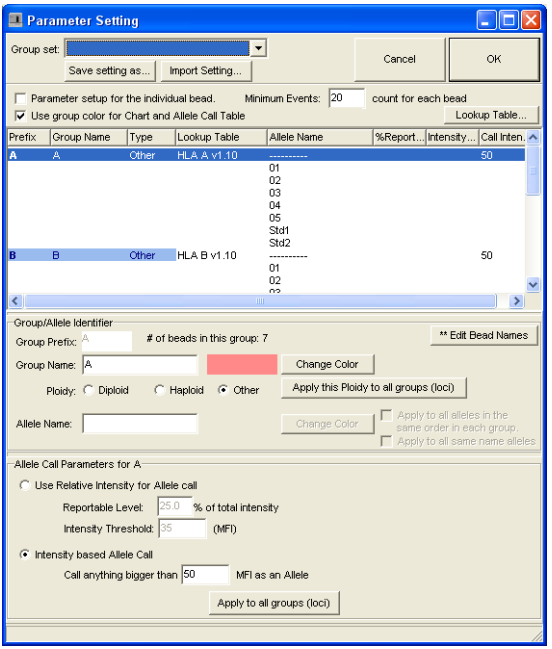


Figure 10.28 Parameter Setting dialog box

3. Click **Lookup Table**.
   ⇒ The Lookup Table Selection window appears (Figure 10.29).

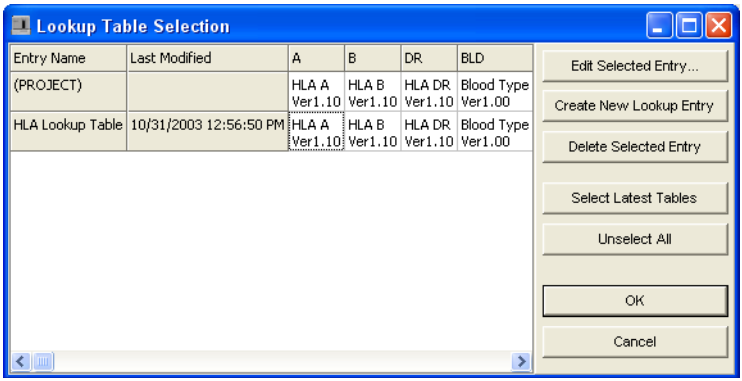


Figure 10.29 Lookup Table Selection window

4. Click the table that you want to edit, and click **Edit Selected Entry**.
   ⇒ The Lookup Table Editor displays the selected table (Figure 10.30).

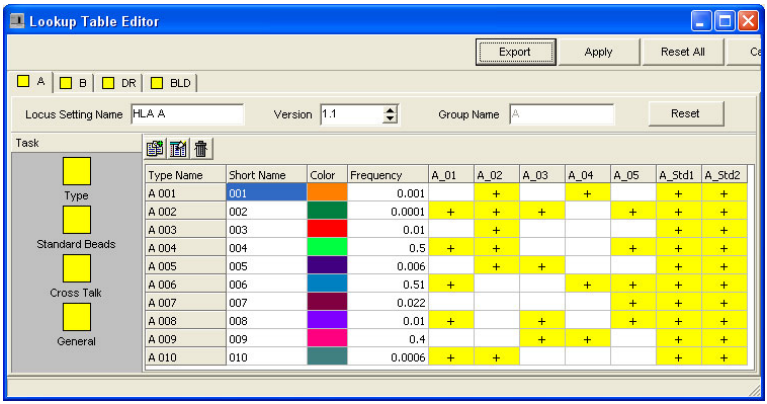


Figure 10.30 Lookup Table Editor

5. Click a locus tab.

7. Click a task: Type, Standard Beads, Cross-talk, or General.

To edit...	Refer to...
Type	*Defining a Type* on page 10.9
Standard Beads	*Setting Standards* on page 10.14
Cross-Talk	*Specifying Cross-talk* on page 10.18
General Parameters	*Setting General Parameters* on page 10.20

8. To return the settings in the current tab to the original values, click **Reset**. To return the settings in all tabs to the original values, click **Reset All**.
9. When you are finished editing the lookup table, click **Apply** and click **Yes** in the confirmation message that appears.

Exporting a Lookup Table

1. Open the project (.gtp) of interest.
2. Click the **Parameter Setting** button  .
   ⇒ The Parameter Setting dialog box appears (Figure 10.31).

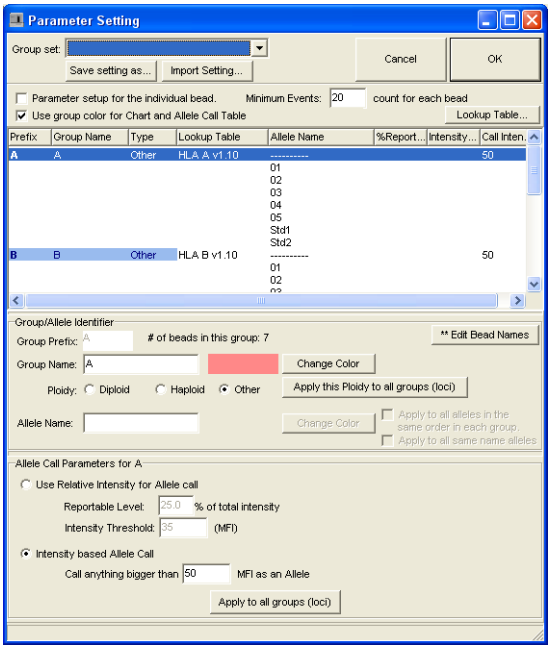


Figure 10.31 Parameter Setting dialog box

3. Click **Lookup Table**.
   ⇒ The Lookup Table Selection window appears (Figure 10.32).

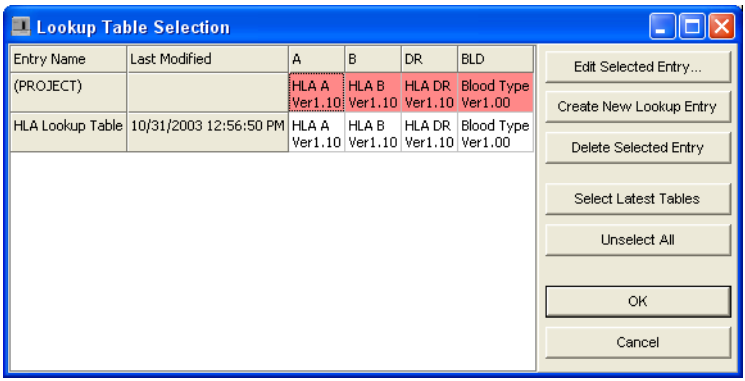


Figure 10.32 Lookup Table Selection window

4. Click the table that you want to export, and click **Edit Selected Entry**.
   ⇒ The Lookup Table Editor appears (Figure 10.33).

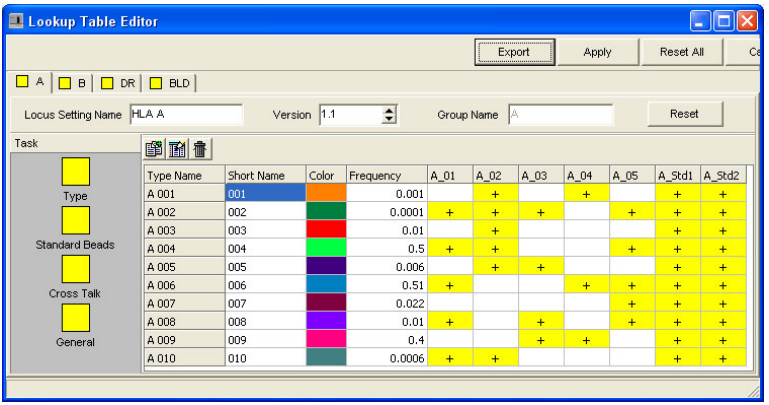


Figure 10.33 Lookup Table Editor

5. Click **Export**.
   ⇒ The Export Wizard appears (Figure 10.34).

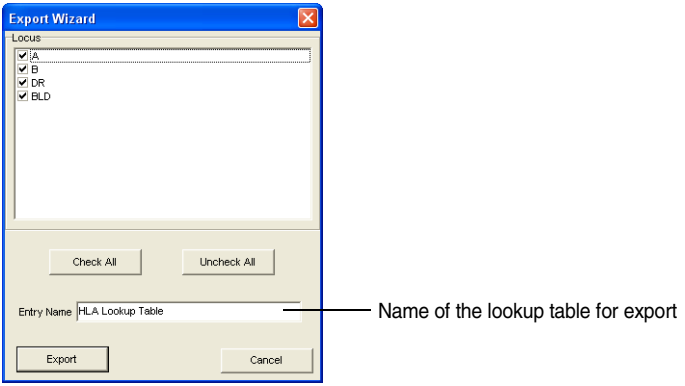


Figure 10.34 Export Wizard

6. To export all of the loci information, click **Check All**. Alternatively, select individual items for export.
7. Click **Export**.
   ⇒ The Save As dialog box appears (Figure 10.35).

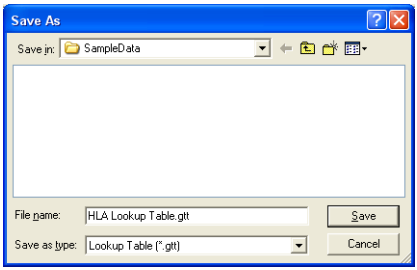


Figure 10.35 Save As dialog box

8. Confirm the destination directory and file name, and click **Save**.
   ⇒ The lookup table (.gtt) is saved to the selected directory.

Copying a Lookup Table

The currently displayed contents of a lookup table can be copied to a tab-delimited text file.

1. Right-click the lookup table and select **Copy as Text** from the shortcut menu that appears.
   ⇒ The contents of the current view of the lookup table are copied to the system clipboard.
2. Paste the clipboard contents to the application of interest (for example, Notepad).

Deleting a Lookup Table

1. Open the Lookup Table Selection window (click  to open the Parameter Setting dialog box and click **Lookup Table**).

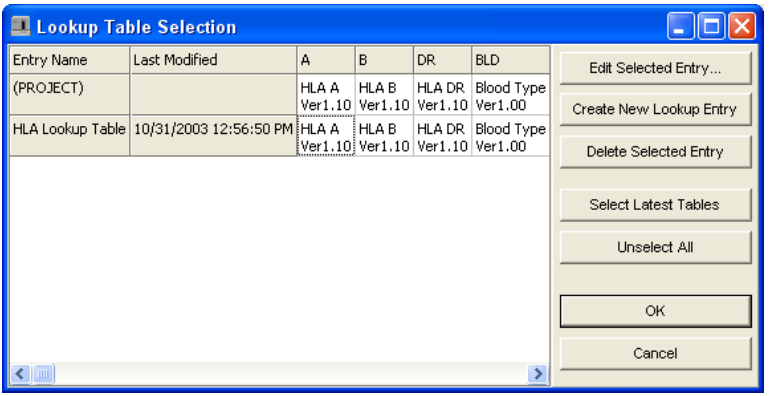


Figure 10.36 Lookup Table Selection window

2. Click the table (row) that you want to delete and click **Delete Selected Entry**. Click **Yes** in the confirmation message that appears.

10.4
HLA Typing Using a Lookup Table

After you open a Luminex® results file (.csv) and import or create a lookup table, the steps to type a sample include:

- Set parameter settings
- Select a lookup table
- View the Typing table

1. To open a Luminex results file (.csv), click the **Open CSV File** button . Alternatively, select **File → Open CSV File** from the menu bar.
   ⇒ The Open dialog box appears (Figure 10.37).
2. Select the .csv of interest and click **Open**.
   ⇒ The Typing table displays the results data.

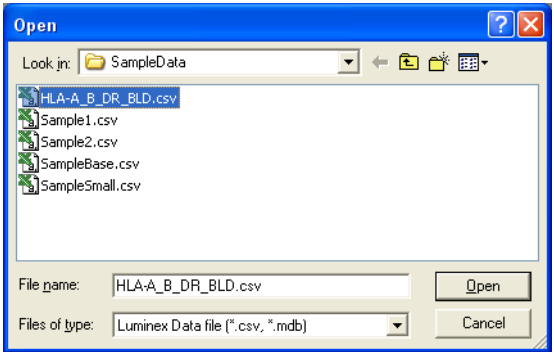


Figure 10.37 Open dialog box

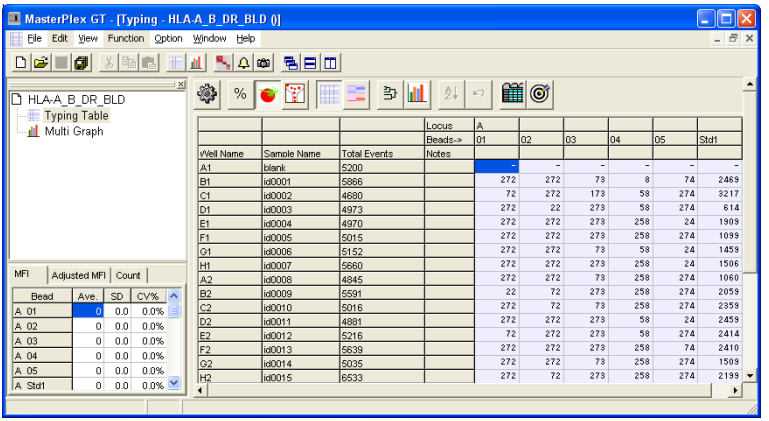


Figure 10.38 MasterPlex GT application window

3. Click the **Parameter Setting** button .
   ⇒ The Parameter Setting dialog box appears (Figure 10.39).

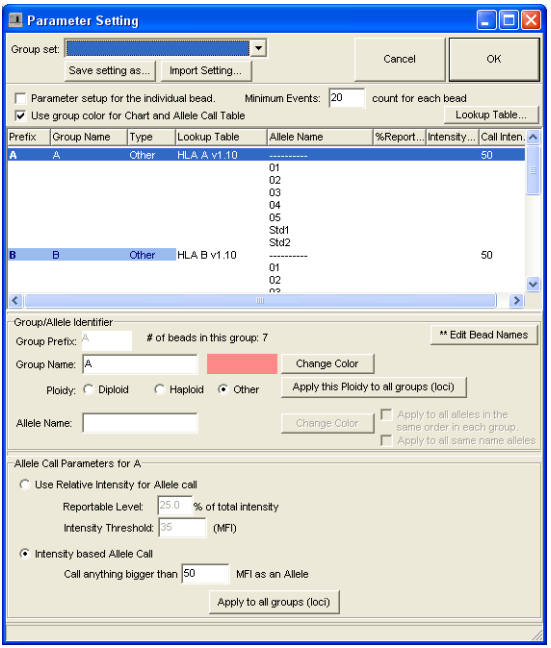


Figure 10.39 Parameter Setting dialog box

4. Choose Intensity Based Allele Call, set the MFI threshold to 50, and click **Apply to all groups (loci)**.
5. In the Parameter Setting dialog box, click **Lookup Table**.
   ⇒ The Lookup Table Selection window appears (Figure 10.40).

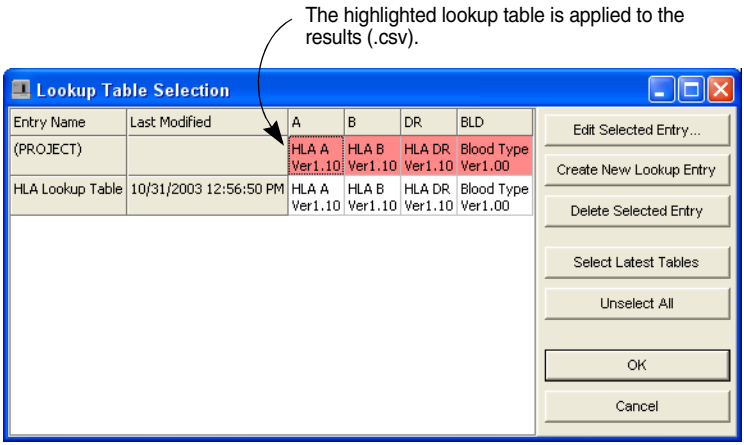


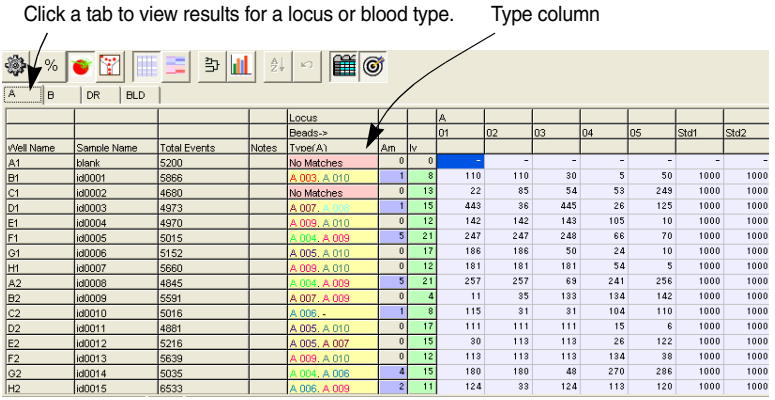
Figure 10.40 Lookup Table Selection window
The window shows the lookup tables that are installed.

6. Click the table that you want to use, and click **OK**.

   The software applies the highlighted table. The newest lookup table version is the default.
7. Click **OK** to close the Parameter Setting dialog box.
   ⇒ The Typing table appears.

NOTE: If a lookup table is not selected, the Type column is empty. To select a look up table, open the Parameter Settings dialog box (click) and click **Lookup Table**.

8. Click the **By Locus** button and **Show Type** button .
   ⇒ The Typing table displays a tab for each locus and the blood type, and a column of genotypes for the selected locus (or blood type) (Figure 10.41).

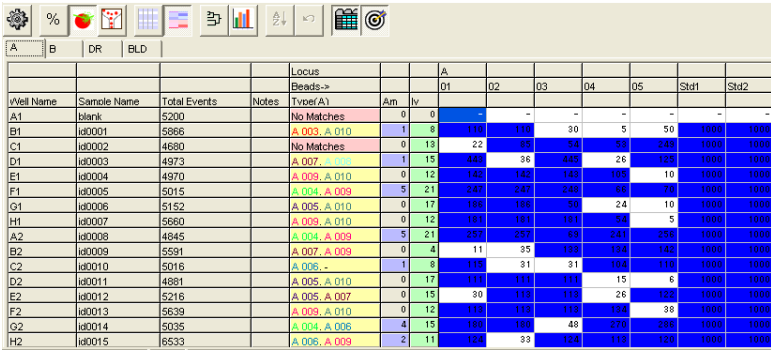


				Locus			A						
				Beads->			01	02	03	04	05	Std1	Std2
Well Name	Sample Name	Total Events	Notes	Type(A)	Am	Iv							
A1	blank	5200		No Matches	0	0	-	-	-	-	-	-	-
B1	id0001	5866		A 003, A 010	1	8	110	110	30	5	50	1000	1000
C1	id0002	4680		No Matches	0	13	22	85	54	53	249	1000	1000
D1	id0003	4973		A 007, A 009	1	15	443	36	445	26	125	1000	1000
E1	id0004	4970		A 009, A 010	0	12	142	142	143	105	10	1000	1000
F1	id0005	5015		A 004, A 009	5	21	247	247	248	66	70	1000	1000
G1	id0006	5152		A 005, A 010	0	17	186	186	50	24	10	1000	1000
H1	id0007	5660		A 009, A 010	0	12	181	181	181	54	5	1000	1000
A2	id0008	4845		A 004, A 009	5	21	257	257	69	241	256	1000	1000
B2	id0009	5591		A 007, A 009	0	4	11	35	133	134	142	1000	1000
C2	id0010	5016		A 006, -	1	8	115	31	31	104	110	1000	1000
D2	id0011	4881		A 005, A 010	0	17	111	111	111	15	6	1000	1000
E2	id0012	5216		A 005, A 007	0	15	30	113	113	26	122	1000	1000
F2	id0013	5639		A 009, A 010	0	12	113	113	113	134	38	1000	1000
G2	id0014	5035		A 004, A 006	4	15	180	180	48	270	286	1000	1000
H2	id0015	6533		A 006, A 009	2	11	124	33	124	113	120	1000	1000

Figure 10.41 Typing table

Type Column Background Color	Indicates...
Red	No matching genotype found.
Yellow	Automatic genotype call.
Blue (Type or Am column)	Ambiguity candidate or number of ambiguity candidates.
Green (Type or Iv column)	Inversion candidate or number of inversion candidates.

10. To view results for another locus or the blood type, click a tab (Figure 10.41).
11. To highlight the samples that exceed the MFI threshold, click the **Gradient Background** button .
    ⇒ Results that exceed the MFI threshold are highlighted (blue in the A locus tab, red in the B locus tab, and alternating red and blue in the remaining tabs) (Figure 10.42).



				Locus			A						
				Beads-»			01	02	03	04	05	Std1	Std2
Well Name	Sample Name	Total Events	Notes	Type(A)	Am	Iv							
A1	blank	5200		No Matches	0	0	-	-	-	-	-	-	-
B1	id0001	5866		A 003, A 010	1	8	119	110	30	5	50	1000	1000
C1	id0002	4680		No Matches	0	13	22	55	52	53	249	1000	1000
D1	id0003	4973		A 007, A 009	1	15	441	36	445	26	125	1000	1000
E1	id0004	4970		A 009, A 010	0	12	141	142	143	105	10	1000	1000
F1	id0005	5015		A 004, A 009	5	21	241	247	248	66	70	1000	1000
G1	id0006	5152		A 005, A 010	0	17	136	138	50	24	10	1000	1000
H1	id0007	5660		A 009, A 010	0	12	131	131	131	30	5	1000	1000
A2	id0008	4845		A 004, A 009	5	21	257	257	258	221	258	1000	1000
B2	id0009	5591		A 007, A 009	0	4	11	95	133	134	142	1000	1000
C2	id0010	5016		A 006, -	1	8	113	31	31	106	110	1000	1000
D2	id0011	4881		A 005, A 010	0	17	111	111	111	15	6	1000	1000
E2	id0012	5216		A 005, A 007	0	15	30	113	113	26	122	1000	1000
F2	id0013	5639		A 009, A 010	0	12	113	113	113	138	38	1000	1000
G2	id0014	5035		A 004, A 006	4	15	180	180	48	270	288	1000	1000
H2	id0015	6533		A 006, A 009	2	11	123	33	123	113	126	1000	1000

Figure 10.42 Typing table, gradient background

The Typing table highlights MFI results that exceed the MFI threshold (set in the Parameter Settings).

12. To view the Allele Call window, right-click the Typing table and select **Allele Call** from the shortcut menu that appears.
    ⇒ The Allele Call window is displayed (Figure 10.43).

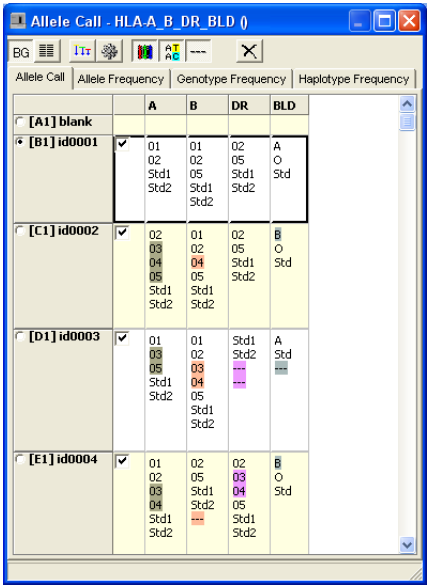
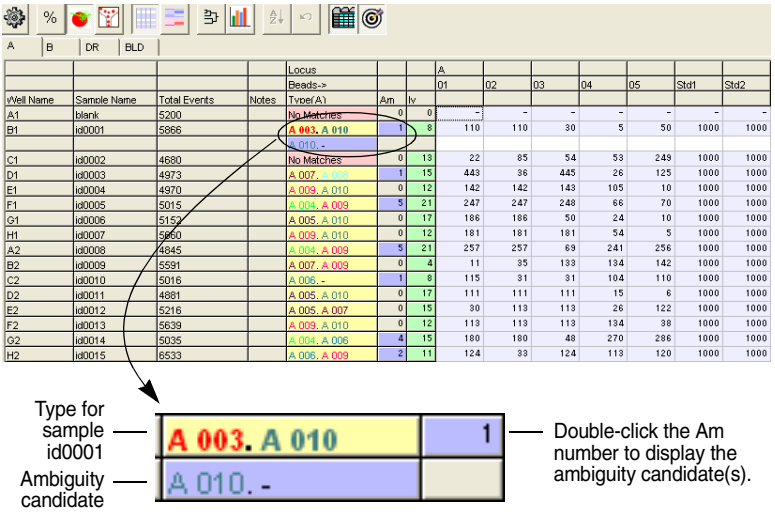


Figure 10.43 Allele Call window

10.5
Ambiguity Candidates

Sometimes there is more than one possible genotype for a sample. This occurs when the allele MFIs that exceed threshold match more than one expression pattern in the lookup table. The Typing table shows the number of ambiguity candidates for a sample in the Am column. If a sample has ambiguity candidates, the Type column shows the genotype with the highest frequency.

- To view the ambiguity candidates for a genotype, double-click the number in the Am column (Figure 10.44). Double-click the number again to collapse the list of ambiguity candidates.



				Locus			A						
				Beads->			01	02	03	04	05	Std1	Std2
Well Name	Sample Name	Total Events	Notes	Type(A)	Am	Iv							
A1	blank	5200		No Matches	0	0	-	-	-	-	-	-	-
B1	id0001	5866		A 003. A 010	1	8	110	110	30	5	50	1000	1000
				A 010. -									
C1	id0002	4680		No Matches	0	13	22	85	54	53	249	1000	1000
D1	id0003	4973		A 007. A 009	1	15	443	36	445	26	125	1000	1000
E1	id0004	4970		A 009. A 010	0	12	142	142	143	105	10	1000	1000
F1	id0005	5015		A 004. A 009	5	21	247	247	248	66	70	1000	1000
G1	id0006	5152		A 005. A 010	0	17	186	186	50	24	10	1000	1000
H1	id0007	5660		A 009. A 010	0	12	181	181	181	54	5	1000	1000
A2	id0008	4845		A 004. A 009	5	21	257	257	69	241	256	1000	1000
B2	id0009	5591		A 007. A 009	0	4	11	35	133	134	142	1000	1000
C2	id0010	5016		A 006. -	1	8	115	31	31	104	110	1000	1000
D2	id0011	4881		A 005. A 010	0	17	111	111	111	15	6	1000	1000
E2	id0012	5216		A 005. A 007	0	15	30	113	113	26	122	1000	1000
F2	id0013	5639		A 009. A 010	0	12	113	113	113	134	38	1000	1000
G2	id0014	5035		A 004. A 006	4	15	180	180	48	270	286	1000	1000
H2	id0015	6533		A 006. A 009	2	11	124	33	124	113	120	1000	1000

Figure 10.44 Typing table
The Am column shows the number of ambiguity candidates for a type. Double-click the Am number to view the ambiguity candidates for a particular type.

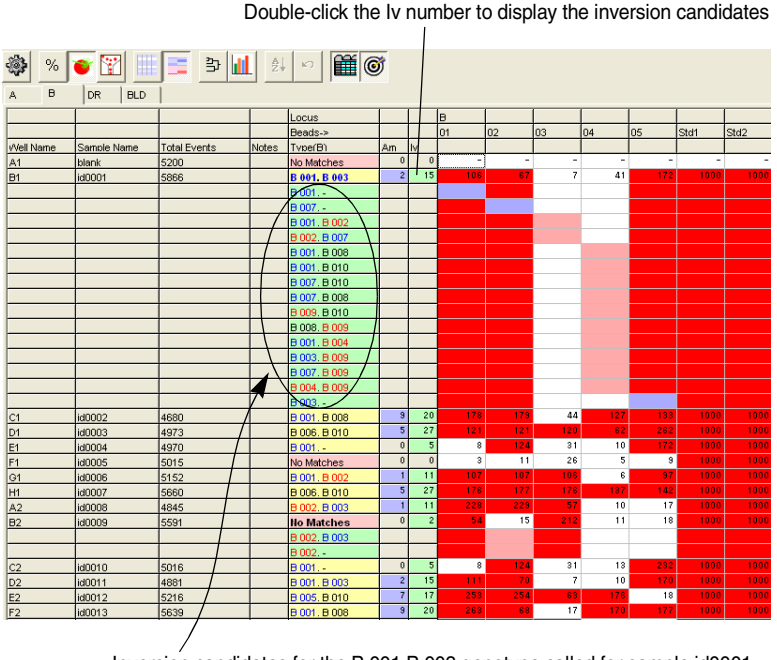
10.6
Inversion Candidates

An inversion candidate is a genotype that is a possible call if the expression level of one allele in the expression pattern is changed to its opposite (for example, an allele that is expressed (MFI > threshold) is changed to not expressed (MFI < threshold). The Typing table shows the inversion candidates for a sample in the Iv column (Figure 10.46).

Checking the inversion candidates is useful way to check for errors, especially for rare combinations of alleles, or other possible genotypes when a no match is called.

To view the inversion candidates for a genotype:

1. Click the **Gradient Background** button  .
   ⇒ Results that exceed the MFI threshold are highlighted (blue in the A locus tab, red in the B locus tab, and alternating red and blue in the remaining tabs) (Figure 10.45).
2. Double-click the number in the Iv column.



				Locus			B						
				Beads-»			01	02	03	04	05	Std1	Std2
Well Name	Sample Name	Total Events	Notes	Type(B)	Am	Iv							
A1	blank	5200		No Matches	0	0	-	-	-	-	-	-	-
B1	id0001	5866		**B 001, B 003**	2	15	105	67	7	41	172	1000	1000
				B 001, -									
				B 007, -									
				B 001, B 002									
				B 002, B 007									
				B 001, B 008									
				B 001, B 010									
				B 007, B 010									
				B 007, B 008									
				B 009, B 010									
				B 008, B 009									
				B 001, B 004									
				B 003, B 009									
				B 007, B 009									
				B 004, B 009									
				B 003, -									
C1	id0002	4680		B 001, B 008	9	20	173	173	44	127	133	1000	1000
D1	id0003	4973		B 006, B 010	5	27	121	121	120	62	262	1000	1000
E1	id0004	4970		B 001, -	0	5	8	124	31	10	172	1000	1000
F1	id0005	5015		No Matches	0	0	3	11	26	5	9	1000	1000
G1	id0006	5152		B 001, B 002	1	11	107	107	106	6	97	1000	1000
H1	id0007	5660		B 006, B 010	5	27	176	177	176	137	142	1000	1000
A2	id0008	4845		B 002, B 003	1	11	229	229	57	10	17	1000	1000
B2	id0009	5591		**No Matches**	0	2	54	15	212	11	18	1000	1000
				B 002, B 003									
				B 002, -									
C2	id0010	5016		B 001, -	0	5	8	124	31	13	232	1000	1000
D2	id0011	4881		B 001, B 003	2	15	111	70	7	10	170	1000	1000
E2	id0012	5216		B 005, B 010	7	17	253	254	68	178	18	1000	1000
F2	id0013	5639		B 001, B 008	9	20	263	68	17	170	177	1000	1000

Figure 10.45 Typing table, gradient background
The Iv column shows the number of inversion candidates for a type. Double-click the Iv number to view the inversion candidates for a particular type.

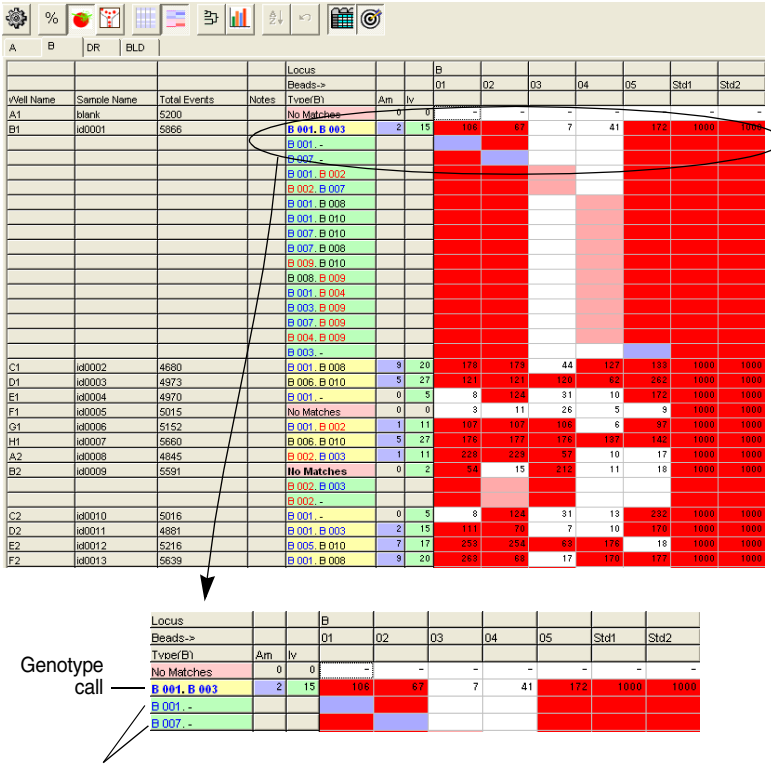


Figure 10.46 Typing table

This chapter explains how to:

- Generate a report
- Preview or print a report
- Save a report.

11.1 The Report Manager

The Report Manager enables you to select the items you want to include in a report and preview the report.

1. To view the Report Manager, click the toolbar button.
   ⇒ The Report Manager appears (Figure 11.1).

   It shows the types of information that can be included in a report.

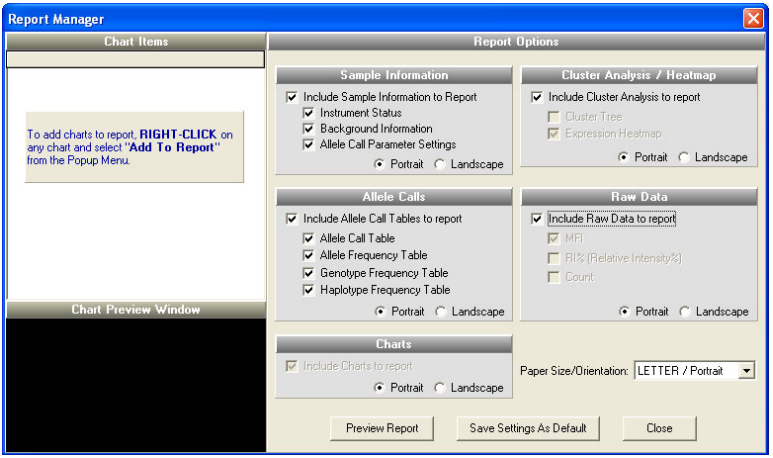


Figure 11.1 Report Manager summarizes report information

2. To remove an item from the report, click the check box to remove the check mark.
3. To include raw data (MFI, percent relative intensity, or bead count) in the report, choose the **Include Raw Data to Report** option.
   ⇒ The current view of the Typing table is added to the report.

4. To specify a paper size and orientation for the report, make a selection from the **Paper Size/Orientation** drop-down list.

   ⇒ The paper size and orientation is set for the report options (sample information, cluster analysis/heat map, allele calls, charts, and raw data).

5. To select the paper orientation for a single report option, click the Portrait or Landscape radio button for the option of interest.

   For example, in Figure 11.2, landscape orientation is chosen for the raw data only.

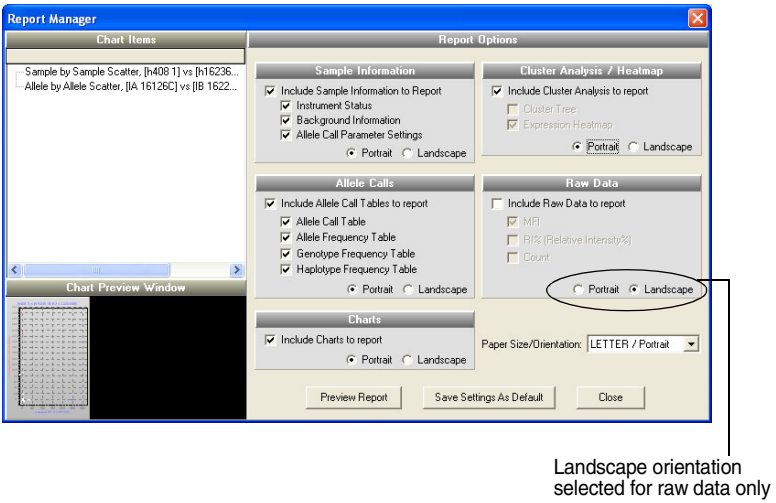


Figure 11.2 Report Manager

6. To save the selected report options as the default, click **Save Settings As Default**.

Adding Charts to a Report

1. In the graph view (click the toolbar button), right-click a chart (graph), and select **Add to Report** from the pop-up menu that appears.

   ⇒ The graph name is added to the chart items list in the Report Manager and the Chart Preview window displays a thumbnail of the chart (Figure 11.3).

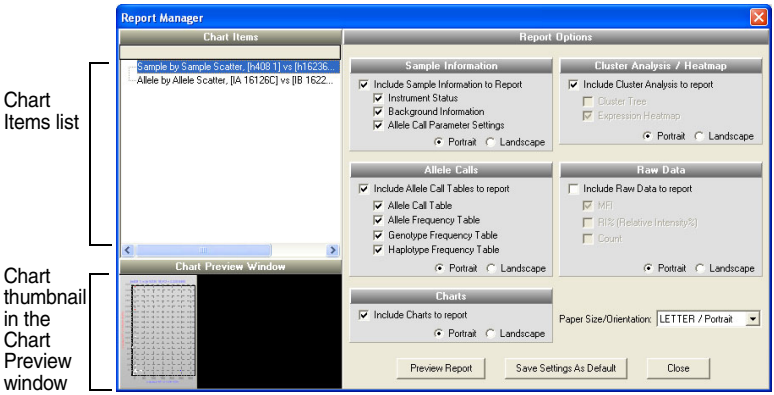


Figure 11.3 Report Manager

2. To add all charts to the report, right-click a chart and select **Add All Charts to Report** from the pop-up menu that appears.
   ⇒ All of the chart names are added to the Chart Items list.
3. To change the display in the Chart Preview window, click the chart of interest in the Chart Items list.
4. To exclude the graphs in the Chart Items list from the report, remove the check mark from the **Include Charts to Report** option.

11.2 Working With a Report in the Preview Window

You can preview a report. In the Preview window, you can:

- print the report
- save the report
- open a report
- perform a text search in the report

Previewing a Report

1. To preview a report, in the Report Manager click **Preview Report** (Figure 11.3).
   ⇒ The Preview window opens and displays the report (Figure 11.4).

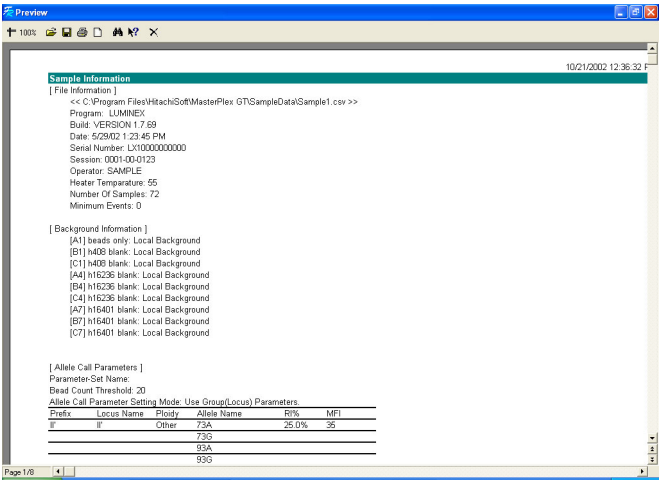


Figure 11.4 Report Preview window

2. To scale the view, click the scale toolbar button 100% and choose a view option.
3. To close the Preview window, click the **Close** button ✕.

Searching a Report

In the Preview window, you can perform a text search in the report.

1. In the Preview window click the **Find text** button 🔭.
   ⇒ The Find text dialog box appears Figure 11.5.

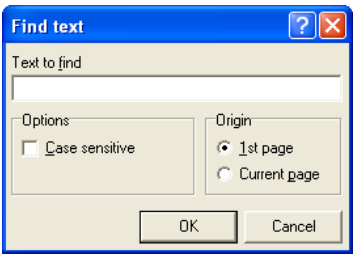


Figure 11.5 Find Text dialog box

2. Enter the text string for the search.

3. If necessary, choose the Case sensitive option.
4. Choose an Origin option for the search: **1st page** starts the search at the first page of the report or **Current page** starts the search at the page currently displayed in the Preview window.
5. Click **OK** to start the search.

Saving a Report

1. In the Preview window, click the **Save** toolbar button .
   ⇒ The Save As dialog box appears (Figure 11.6).

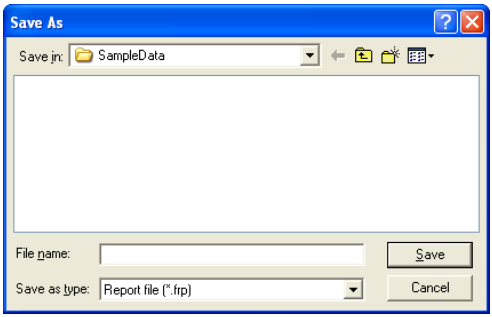


Figure 11.6 Save As dialog box

2. Select a directory and enter a name for the report (.rpt).
3. Click **Save**.

Printing a Report

1. In the Preview window, click the **Print** toolbar button .
   ⇒ The Print dialog box appears (Figure 11.7).

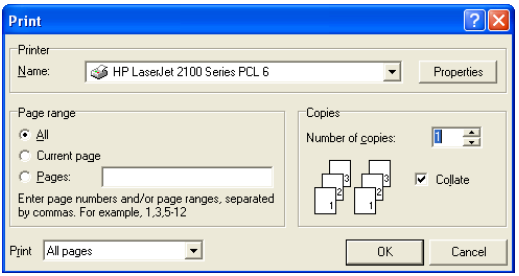


Figure 11.7 Print dialog box

2. Specify a print range and the number of copies.
3. Click **OK** to print the report.

11.3 Opening a Report

1. Open the Report Manager (click the toolbar button).
2. Click the **Preview Report**.
   ⇒ The Preview window opens (Figure 11.8).

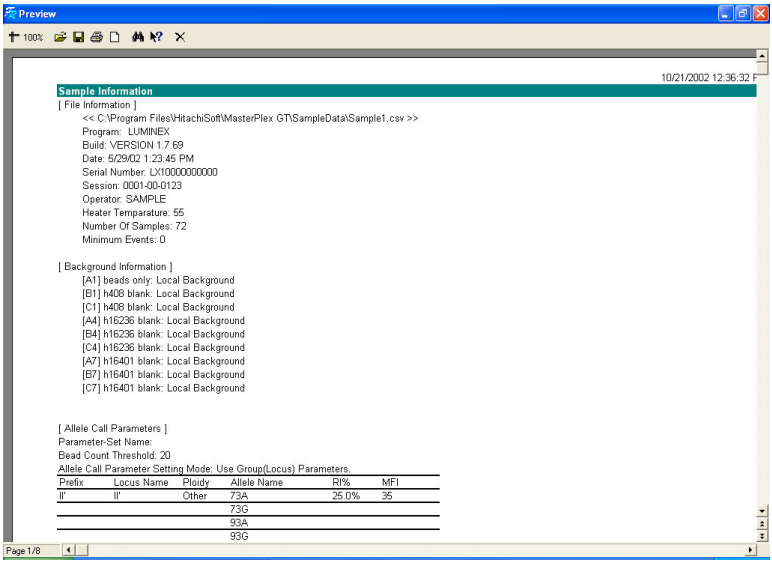


Figure 11.8 Preview window

3. Click the **Open Report** toolbar button .
   ⇒ The Open dialog box appears (Figure 11.9).

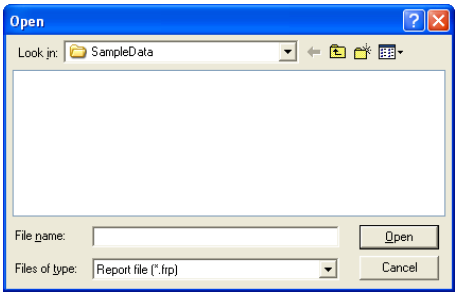


Figure 11.9 Open dialog box

4. Specify a directory and enter the report name (.frp).
5. Click **Open**.
   ⇒ The report is displayed in the Preview window.

APPENDIX A APPLICATION OPTIONS

The application options are user-modifiable settings that are applied to all open results files (.csv) or projects (.gtp). This appendix explains the types of options available.

A.1 General Options

1. To view the general options, select **Options → Set Application Options** from the menu bar.

   ⇒ The Application Options dialog box appears (Figure A.1).

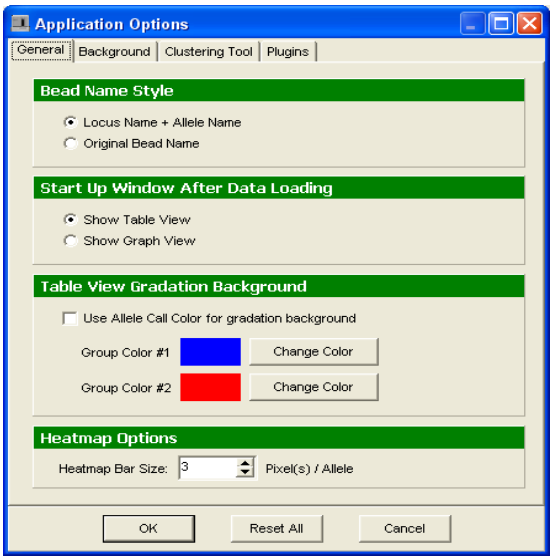


Figure A.1 Application Options, General tab

In the General tab (Figure A.1), the user-modifiable settings include:

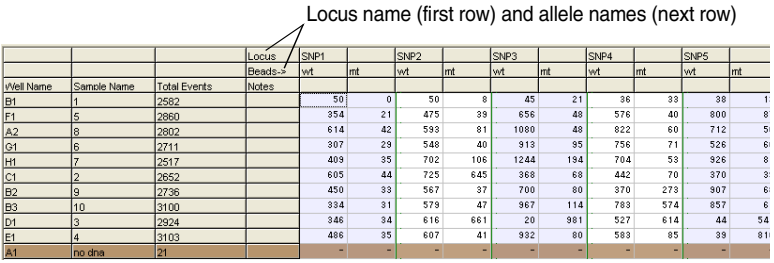
- **Bead Name Style** Specifies how the bead set names are displayed in the Typing table. Choose the locus and allele names or the names entered in the Luminex® system.
- **Start Up Window After Data Loading** Determines whether the Project Window displays the Typing table or the Multi Graph view upon opening a results file (.csv) or a project (.gtp).

- **Table View Gradation Background** Enables you to choose different gradient background colors for the Typing table.
- **Heat map Options** Enable you to change the width of the bars in the map.

Bead Name Style

You can specify how to display the bead names in the Typing table (Figure A.1). Choose one of the options:

- **Locus Name + Allele Name** displays the locus (group) name and the allele name for each bead type (Figure A.2)
- **Original Bead Name** displays the bead name entered in the Luminex® system (Figure A.3).



			Locus	SNP1		SNP2		SNP3		SNP4		SNP5	
			Beads->	wt	mt	wt	mt	wt	mt	wt	mt	wt	mt
Well Name	Sample Name	Total Events	Notes										
B1	1	2582		50	0	50	8	45	21	36	33	38	1
F1	5	2860		354	21	475	39	656	48	576	40	800	8
A2	8	2802		614	42	593	81	1080	48	822	60	712	5
G1	6	2711		307	29	548	40	913	95	756	71	526	6
H1	7	2517		409	35	702	106	1244	194	704	53	926	8
C1	2	2652		605	44	725	645	368	68	442	70	370	3
B2	9	2736		450	33	567	37	700	80	370	273	907	6
B3	10	3100		334	31	579	47	967	114	783	574	857	6
D1	3	2924		346	34	616	661	20	981	527	614	44	54
E1	4	3103		486	35	607	41	932	80	583	85	39	81
A1	no dna	21		-	-	-	-	-	-	-	-	-	

Figure A.2 Typing table, Locus Name + Bead Name option selected

			Locus										
			Beads->	SNP1 wt	SNP1 mt	SNP2 wt	SNP2 mt	SNP3 wt	SNP3 mt	SNP4 wt	SNP4 mt	SNP5 wt	SNP5 mt
Well Name	Sample Name	Total Events	Notes										
B1	1	2582		50	0	50	8	45	21	36	33	38	13
F1	5	2860		354	21	475	39	656	48	576	40	800	87
A2	8	2802		614	42	593	81	1080	48	822	60	712	50
G1	6	2711		307	29	548	40	913	95	756	71	526	60
H1	7	2517		409	35	702	106	1244	194	704	53	926	81
C1	2	2652		605	44	725	645	368	68	442	70	370	39
B2	9	2736		450	33	567	37	700	80	370	273	907	68
B3	10	3100		334	31	579	47	967	114	783	574	857	61
D1	3	2924		346	34	616	661	20	981	527	614	44	541
E1	4	3103		486	35	607	41	932	80	583	85	39	810
A1	no dna	21		-	-	-	-	-	-	-	-	-	-

Figure A.3 Typing table, Original Bead Name option selected

Start Up Window After Data Loading

The Project Window can display the Typing table or Multi Graph view when you open a results file (.csv) or a project (.gtp).

Choose:

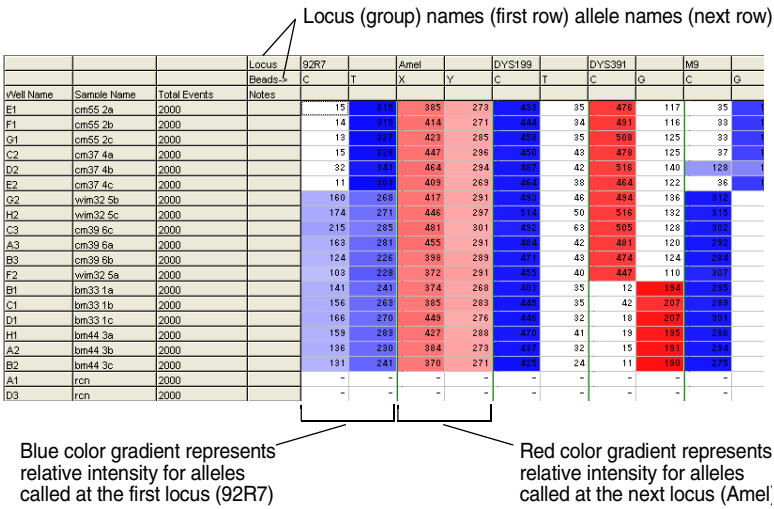
- **Show Table View** to display the Typing table when the Project Window opens.

- **Show Graph View** to display the Multi Graph view when the Project Window opens.

Table View Gradation Background

The Typing table with the gradient background (Figure A.4) uses a color gradient to indicate the relative expression level of the alleles called at each locus in a sample (a lighter shade represents a lower expression level). Alternating colors (defaults are blue and red) distinguish the alleles (rows) of adjacent loci (groups) in the table.

For example, in Figure A.4, the alleles of the first locus (92R7) are highlighted with a blue color gradient that represents the relative expression levels of the called alleles (a lighter color shade indicates a lower expression level). In the next locus (Amel), a red color gradient highlights the relative expression levels of the called alleles. Alternating blue and red color gradients represent the relative expression levels of the remaining loci in the table.



			Locus	92R7		Amel		DYS199		DYS391		M9	
			Beads->	C	T	X	Y	C	T	C	G	C	G
Well Name	Sample Name	Total Events	Notes										
E1	cm55 2a	2000		15	313	385	273	438	35	476	117	35	
F1	cm55 2b	2000		14	313	414	271	444	34	491	116	33	
G1	cm55 2c	2000		13	327	423	285	458	35	508	125	33	
C2	cm37 4a	2000		15	328	447	296	450	43	478	125	37	
D2	cm37 4b	2000		32	341	464	294	487	42	516	140	128	
E2	cm37 4c	2000		11	301	409	269	464	38	464	122	36	
G2	wim32 5b	2000		160	268	417	291	493	46	494	136	312	
H2	wim32 5c	2000		174	271	446	297	514	50	516	132	315	
C3	cm39 6c	2000		215	285	481	301	492	63	505	128	302	
A3	cm39 6a	2000		163	281	455	291	484	42	481	120	292	
B3	cm39 6b	2000		124	226	398	289	471	43	474	124	284	
F2	wim32 5a	2000		103	228	372	291	455	40	447	110	307	
B1	bm33 1a	2000		141	241	374	268	401	35	12	194	295	
C1	bm33 1b	2000		156	263	385	283	445	35	42	207	283	
D1	bm33 1c	2000		166	270	449	276	448	32	18	207	301	
H1	bm44 3a	2000		159	283	427	288	470	41	19	195	286	
A2	bm44 3b	2000		136	230	384	273	437	32	15	191	294	
B2	bm44 3c	2000		131	241	370	271	425	24	11	190	275	
A1	rcn	2000		-	-	-	-	-	-	-	-	-	
D3	rcn	2000		-	-	-	-	-	-	-	-	-	

Figure A.4 Typing table, gradient background
The default gradient background (alternating red and blue) shows relative expression levels of the alleles called in each sample.

Changing the Gradient Background Colors

You can change the default gradient background colors in the Typing table or you can apply the group colors (specified in the Parameter Setting dialog box) to the Typing table.

NOTE: When you change the Group #1 or #2 color, the new color is also applied to the graph points in the Sample by Sample scatter graph and the x-axis and y-axis thresholds in the Allele by Allele scatter graph.

To select a different Group #1 or Group #2 color:

1. In the Applications Options dialog box (Figure A.1), click **Change Color** for Group #1 (or Group #2).
   ⇒ The color palette appears (Figure A.5).

Figure A.5 Color palette

2. To select a predefined color, click one of the basic colors.
3. To define a custom color, click **Define Custom Colors**.
   ⇒ The color palette shows the custom color options (Figure A.6).

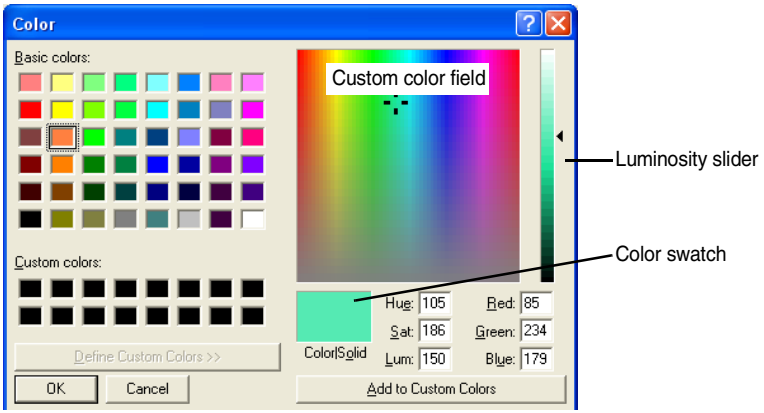


Figure A.6 Color palette, custom color options

4. To define a color, use the click-and-drag operation to move the cross hairs in the custom color field. Adjust the color brightness using the luminosity slider.

   ⇒ The Color swatch shows the color selection.

5. When you are finished defining the color, click **Add to Custom Colors** to apply the color, and click **OK**.

   ⇒ The new color is displayed in the Applications Options dialog box.

6. Click **OK** in the Applications Options dialog box to close the dialog box and apply the color to the Typing table gradient background.
7. To apply the group colors to the Typing table, choose the **Use Allele Call Color for Gradation Background** option (Figure A.7).

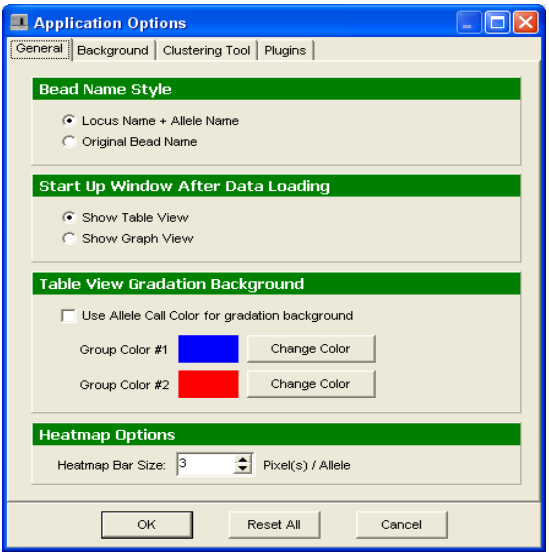


Figure A.7 Application Options, General tab

Heat Map Options

The default bar width is 6 pixels. To change the bar width, make a selection from the Pixels/allele drop-down list (1 pixel = minimum width, 10 pixel = maximum width).

A.2
Background Options

The MasterPlex™ GT software can automatically identify the negative controls in a results file (.csv) by searching for key words in the sample name. In the Background tab of the Application dialog box, you can set the key words that identify a negative control.

1. Select **Option Set → Application Options** from the menu bar.
   ⇒ The Application Options dialog box opens (Figure A.8).

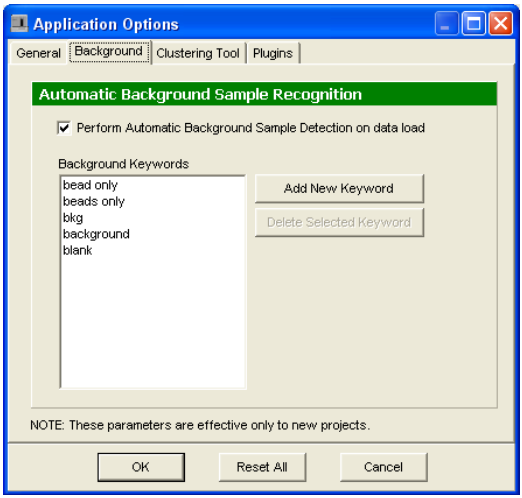


Figure A.8 Application Options, Background tab

2. Click the Background tab.
3. Choose the option **Perform Automatic Background Sample Detection on data load**.
4. To define a keyword, click **Add New Keyword,** enter the keyword(s) in the dialog box that appears, and click **OK** (Figure A.9).

   ⇒ The keyword is added to the Background Keywords list (Figure A.8).

NOTE: A keyword added during a session is applied only to subsequently opened results files (.csv) or projects (.gtp).

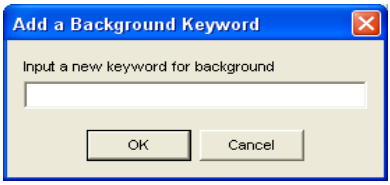


Figure A.9 Add a Background Keyword dialog box

5. To delete a keyword, select the keyword you want to delete in the Background Keywords list and click **Delete Selected Keyword**. At the prompt, click **Yes**.

A.3
Clustering Tool Options

You can have the Clustering Tool window (Figure A.10) open in the Multi Graph view when you work with the Multi Compare, Depth, or Sample by Sample scatter graph. You can adjust the transparency of the Clustering Tool window.

NOTE: This option is only available for the Windows™ 2000 or XP operating system.

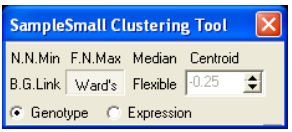


Figure A.10 Clustering Tool window

1. Select **Option Set → Application Options** from the menu bar.
   ⇒ The Application Options dialog box opens ((Figure A.11)).

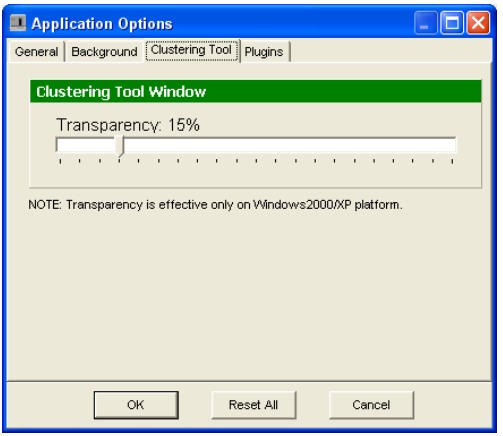


Figure A.11 Application Options, Clustering Tool tab

2. Click the Clustering Tool tab.
3. To increase or decrease the Clustering Tool window transparency, click and move the slider to the right or left.

A.4
Plug-ins

This tab shows plug-in applications that are available to the MasterPlex™ GT software.

1. Select **Option Set → Application Options** from the menu bar.
   ⇒ The Application Options dialog box opens ((Figure A.12)).

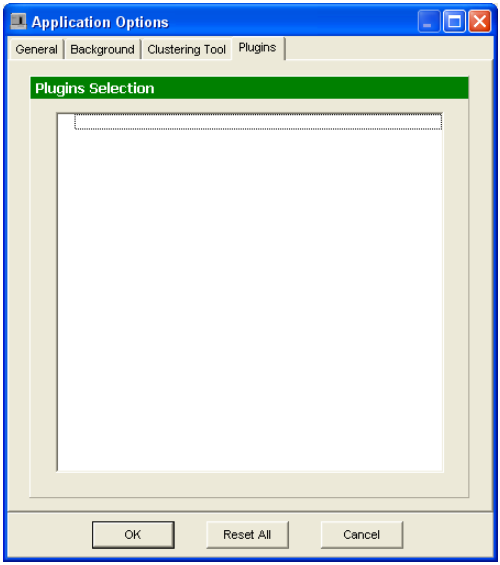


Figure A.12 Application Options, Plug-in tab

2. Click the Plug-in tab.
3. Place a check mark next to the plug-in application that you want to use with the MasterPlex™ GT software.

   The plug-in application automatically starts the next time MasterPlex GT is started.

4. To disable a plug-in application, remove the check mark next to the it.

A.5
Resetting the Application Options

To return all user-modifiable settings to the factory set defaults, click **Reset All** (Figure A.12).

APPENDIX B PROJECT OPTIONS & PARAMETERS

The project options and user-modifiable parameters are the default settings that the MasterPlex™ GT software applies when you open a results file(s) (.csv) and start a new project. You can set defaults for the:

- allele calling algorithm
- Typing table view
- Multi Compare and Depth bar graph display
- Allele Call table view
- cluster analysis tool

These settings apply only to new projects. This appendix explains the types of options available to you and the user-modifiable parameters for new projects.

To view the project options and parameters:

1. Select **Option → Set New Project Default Parameters** from the menu bar.
   ⇒ The New Project Default Parameters dialog box opens (Figure B.1).

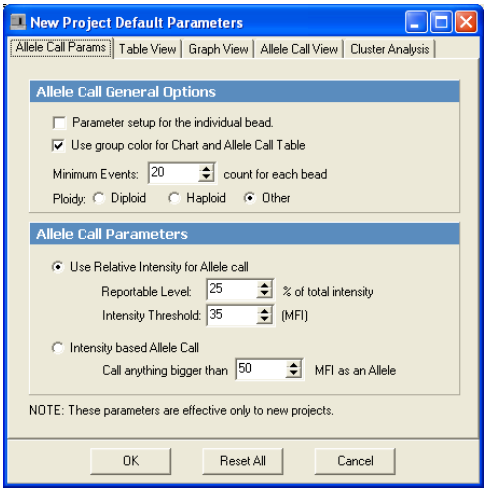


Figure B.1 New Project Default Parameters dialog box, Allele Call Parameters tab

B.1
Allele Call Parameters

In the Allele Call Parameters tab (Figure B.1) you can specify defaults for the Parameter Settings dialog box (Figure B.2).

Allele Call General Options

Parameter setup for the individual bead Choose this option to specify allele call parameters for an individual bead type.

Use group color for Chart and Allele Call table Choose this option to display the group colors (specified in the Parameter Settings dialog box) in the Allele Call table, Multi Compare graph, and Depth graph.

Ploidy Choose a ploidy option for the sample data. (Note: 'Other' ploidy is the same as haploid.) The type of ploidy affects the allele frequency calculation.

Allele Call Parameters

Use Relative Intensity for Allele Call The software calls the allele if all of the following conditions are met:

- $RI_{allele} \geq$ user-specified RI threshold
- $MFI_{allele} \geq$ user-specified intensity threshold

Intensity Based Allele Call The software calls the allele if the $MFI_{allele} >$ user-specified absolute intensity threshold

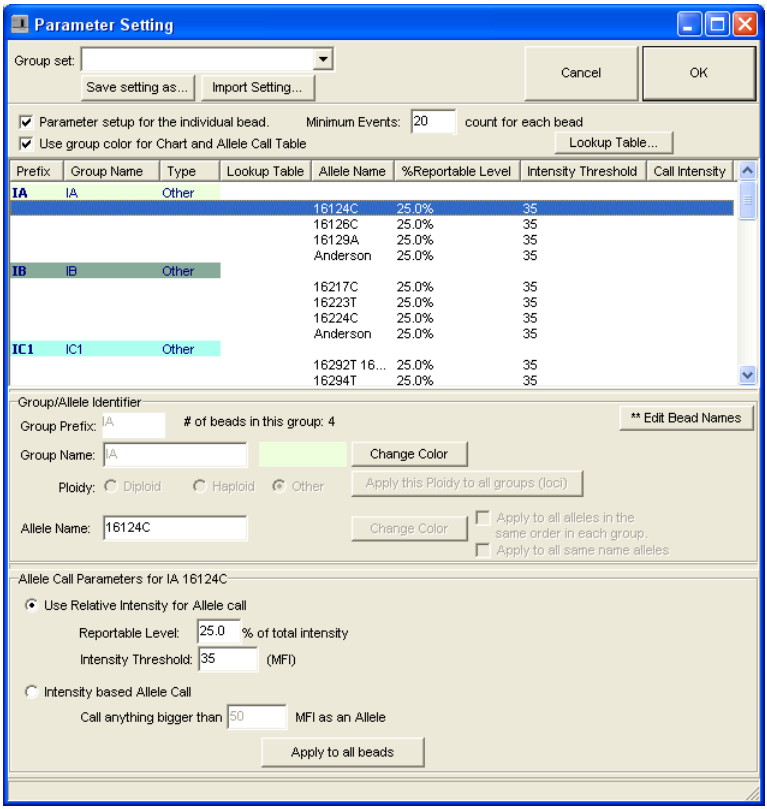


Figure B.2 Parameter Setting dialog box

B.2
Table View

In the Table View tab (Figure B.3) you can specify display defaults for the Typing table.

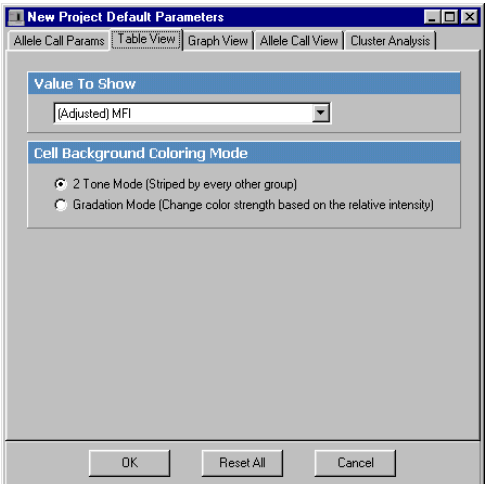


Figure B.3 New Project Default parameters dialog box, Table View tab

Value to Show

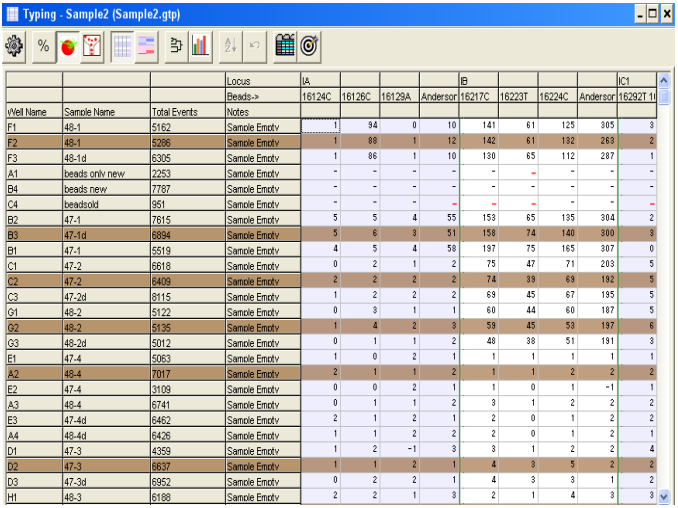
Make a selection from the drop-down list to specify the default Typing table view that is displayed when you open a results file (.csv) or project (.gtp). Choose from the following data formats:

- percent relative intensity
- background-adjusted MFI
- bead count

Cell Background Coloring Mode

2 Tone Mode Choose this option to use alternating colors to distinguish loci (groups) in the Typing table (Figure B.4).

Gradation Mode Choose this option to use a color gradient to indicate relative percent intensity of the alleles in a group (Figure B.5).



			Locus	IA				IB				IC1
			Beads-»	16124C	16126C	16129A	Andersor	16217C	16223T	16224C	Andersor	16292T 1(
Well Name	Sample Name	Total Events	Notes									
F1	48-1	5162	Sample Empty	1	94	0	10	141	61	125	305	3
F2	48-1	5286	Sample Empty	1	88	1	12	142	61	132	263	2
F3	48-1d	6305	Sample Empty	1	86	1	10	130	65	112	287	1
A1	beads only new	2253	Sample Empty	-	-	-	-	-	-	-	-	-
B4	beads new	7787	Sample Empty	-	-	-	-	-	-	-	-	-
C4	beadsold	951	Sample Empty	-	-	-	-	-	-	-	-	-
B2	47-1	7615	Sample Empty	5	5	4	55	153	65	135	304	2
B3	47-1d	6894	Sample Empty	5	6	3	51	158	74	140	300	3
B1	47-1	5519	Sample Empty	4	5	4	58	197	75	165	307	0
C1	47-2	6618	Sample Empty	0	2	1	2	75	47	71	203	5
C2	47-2	6409	Sample Empty	2	2	2	2	74	39	69	192	5
C3	47-2d	8115	Sample Empty	1	2	2	2	69	45	67	195	5
G1	48-2	5122	Sample Empty	0	3	1	1	60	44	60	187	5
G2	48-2	5135	Sample Empty	1	4	2	3	59	45	53	197	6
G3	48-2d	5012	Sample Empty	0	1	1	2	48	38	51	191	3
E1	47-4	5063	Sample Empty	1	0	2	1	1	1	1	1	1
A2	48-4	7017	Sample Empty	2	1	1	2	1	1	2	2	2
E2	47-4	3109	Sample Empty	0	0	2	1	1	0	1	-1	1
A3	48-4	6741	Sample Empty	0	1	1	2	3	1	2	2	2
E3	47-4d	6462	Sample Empty	2	1	2	1	2	0	1	2	2
A4	48-4d	6426	Sample Empty	1	1	2	2	2	0	1	2	1
D1	47-3	4359	Sample Empty	1	2	-1	3	3	1	2	2	4
D2	47-3	6637	Sample Empty	1	1	2	1	4	3	5	2	2
D3	47-3d	6952	Sample Empty	0	2	2	1	4	3	3	1	2
H1	48-3	6188	Sample Empty	2	2	1	3	2	1	4	3	3

Figure B.4 Typing table, two tone mode (stripe background)

			Locus	IA				IB			
			Beads->	16124C	16126C	16129A	Andersor	16217C	16223T	16224C	Andersor
Well Name	Sample Name	Total Events	Notes								
F1	48-1	5162	Sample Emptv	1	84	0	10	141	61	125	305
F2	48-1	5286	Sample Emptv	1	88	1	12	142	61	132	263
F3	48-1d	6305	Sample Emptv	1	86	1	10	130	65	112	287
A1	beads only new	2253	Sample Emptv	-	-	-	-	-	-	-	-
B4	beads new	7787	Sample Emptv	-	-	-	-	-	-	-	-
C4	beadsold	951	Sample Emptv	-	-	-	-	-	-	-	-
B2	47-1	7615	Sample Emptv	5	5	4	59	153	65	135	304
B3	47-1d	6894	Sample Emptv	5	6	3	51	158	74	140	300
B1	47-1	5519	Sample Emptv	4	5	4	58	197	75	165	307
C1	47-2	6618	Sample Emptv	0	2	1	2	75	47	71	203
C2	47-2	6409	Sample Emptv	2	2	2	2	74	39	69	192
C3	47-2d	8115	Sample Emptv	1	2	2	2	69	45	67	195
G1	48-2	5122	Sample Emptv	0	3	1	1	60	44	60	187
G2	48-2	5135	Sample Emptv	1	4	2	3	59	45	53	197
G3	48-2d	5012	Sample Emptv	0	1	1	2	48	38	51	191
E1	47-4	5063	Sample Emptv	1	0	2	1	1	1	1	1
A2	48-4	7017	Sample Emptv	2	1	1	2	1	1	2	2
E2	47-4	3109	Sample Emptv	0	0	2	1	1	0	1	-1
A3	48-4	6741	Sample Emptv	0	1	1	2	3	1	2	2
E3	47-4d	6462	Sample Emptv	2	1	2	1	2	0	1	2
A4	48-4d	6426	Sample Emptv	1	1	2	2	2	0	1	2
D1	47-3	4359	Sample Emptv	1	2	-1	3	3	1	2	2
D2	47-3	6637	Sample Emptv	1	1	2	1	4	3	5	2
D3	47-3d	6952	Sample Emptv	0	2	2	1	4	3	3	1
H1	48-3	6188	Sample Emptv	2	2	1	3	2	1	4	3

Figure B.5 Typing table, gradient background

B.3
Graph View

The settings in the Graph View tab (Figure B.6) specify the defaults for the Multi Compare and Depth graph display.

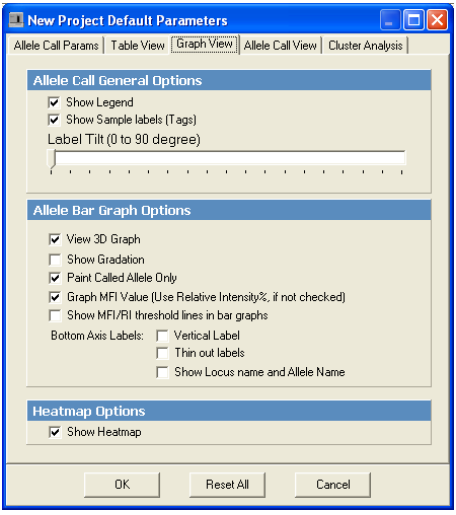


Figure B.6 New Project Default Parameters, Graph View tab

Allele Call General Options

Show Legend	Choose this option to display the allele name legend in the Multi Compare graph.
Show Sample Labels (Tags)	Choose this option to display the allele names in the Multi Compare and Depth bar graphs (Figure B.7).
Label Tilt	Move the slider to the right to rotate the allele name labels counter clockwise in the Multi Compare graph.

Allele Bar Graph Options

These are display options for the bars that represent alleles in the Multi Compare and Depth bar graphs.

Option	Choose this option to...
View 3D Graph	Display three-dimensional bars in the Multi Compare graph (Figure B.7).
Show Gradation	Display the Multi Compare and Depth graph bars using a color gradient (Figure B.8).
Paint Called Allele Only	Apply the group color only to the bars that represent called alleles in the Multi Compare and Depth bar graphs and the graph legends (Figure B.9).
Graph MFI Value (Use Relative Intensity %, if not checked)	Plot background-adjusted MFI data in the Multi Compare and Depth bar graphs. If this option is not chosen, the bar graphs plot percent relative intensity (Figure B.10).
Show MFI/RI threshold lines in bar graphs	Display the threshold lines in the Multi Compare bar graphs (Figure B.7).
Vertical Label	Display labels vertically along the x-axis of the graphs.
Thin out labels	Show a subset of the labels along the x-axis of the graph so that none of the labels overlap.
Show Locus Name and Allele Name	Show both the locus (group) and allele name on the x-axis of the graph.
Show Heat map	Display the Heat map in the Multi Graph view.

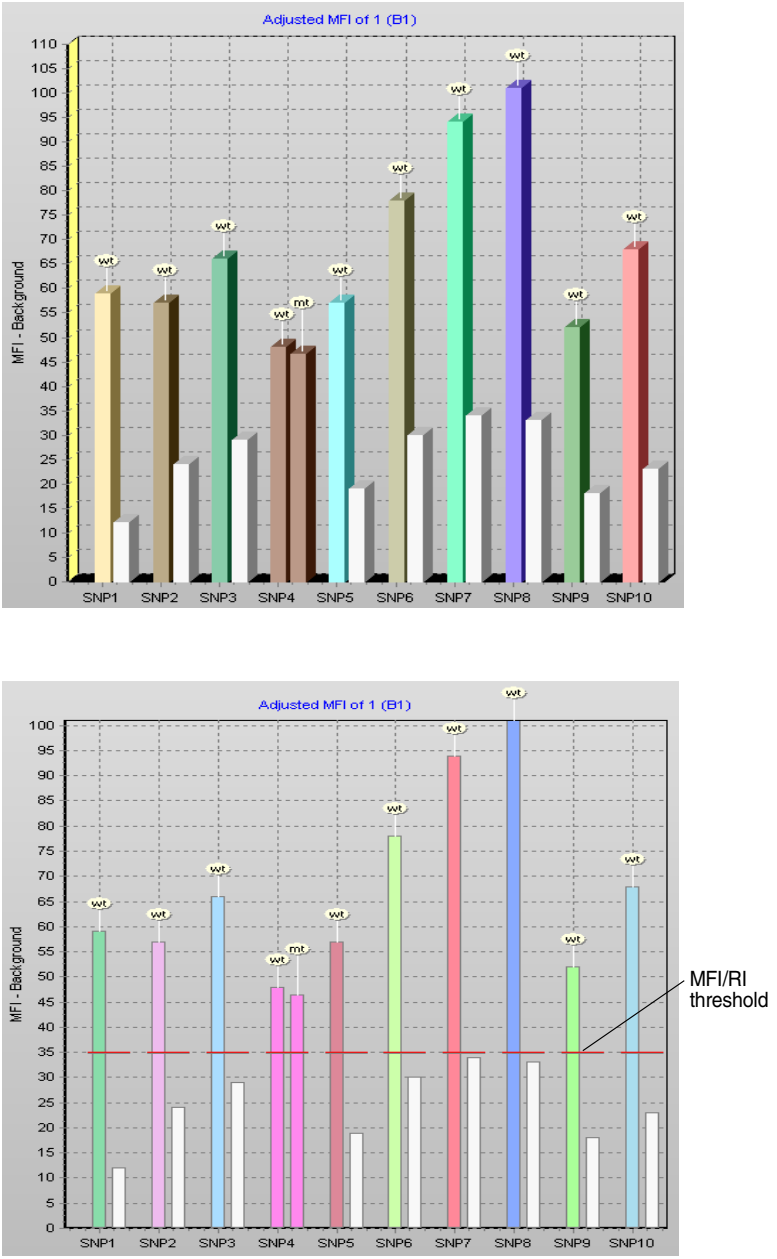


Figure B.7 Multi Compare graph, 3D bars (top), 2D bars (bottom)

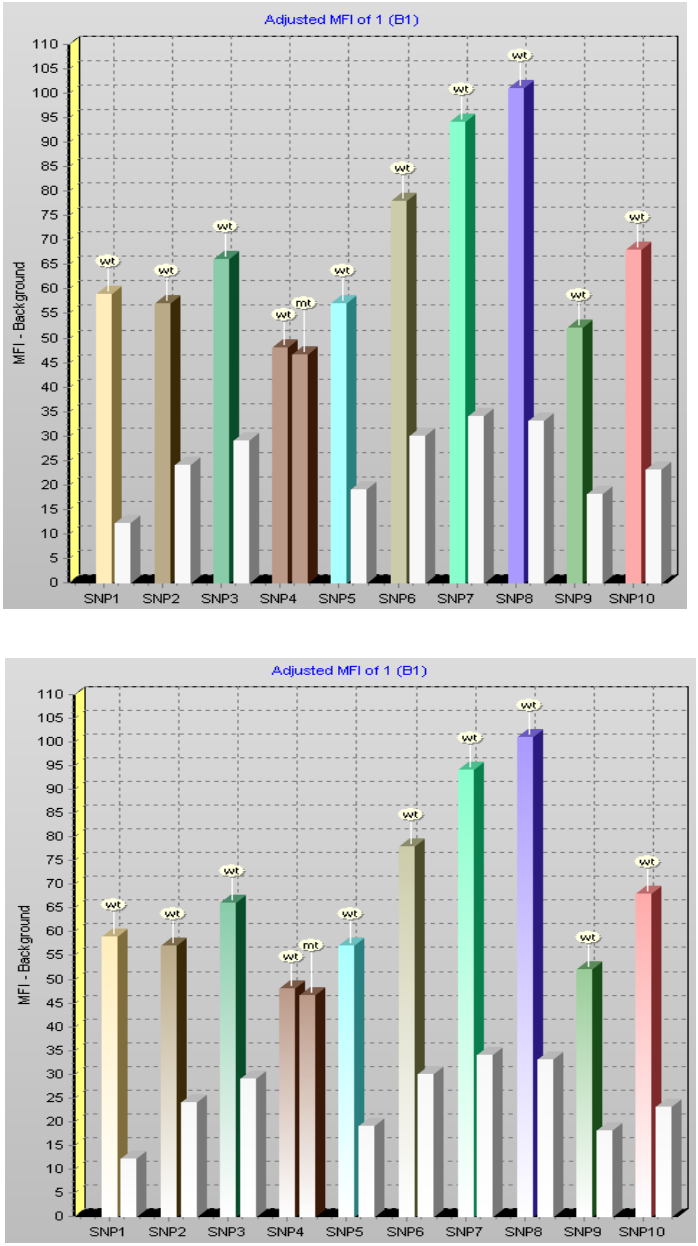


Figure B.8 Multi Compare graph, solid color bars (top), gradient color bars (bottom)

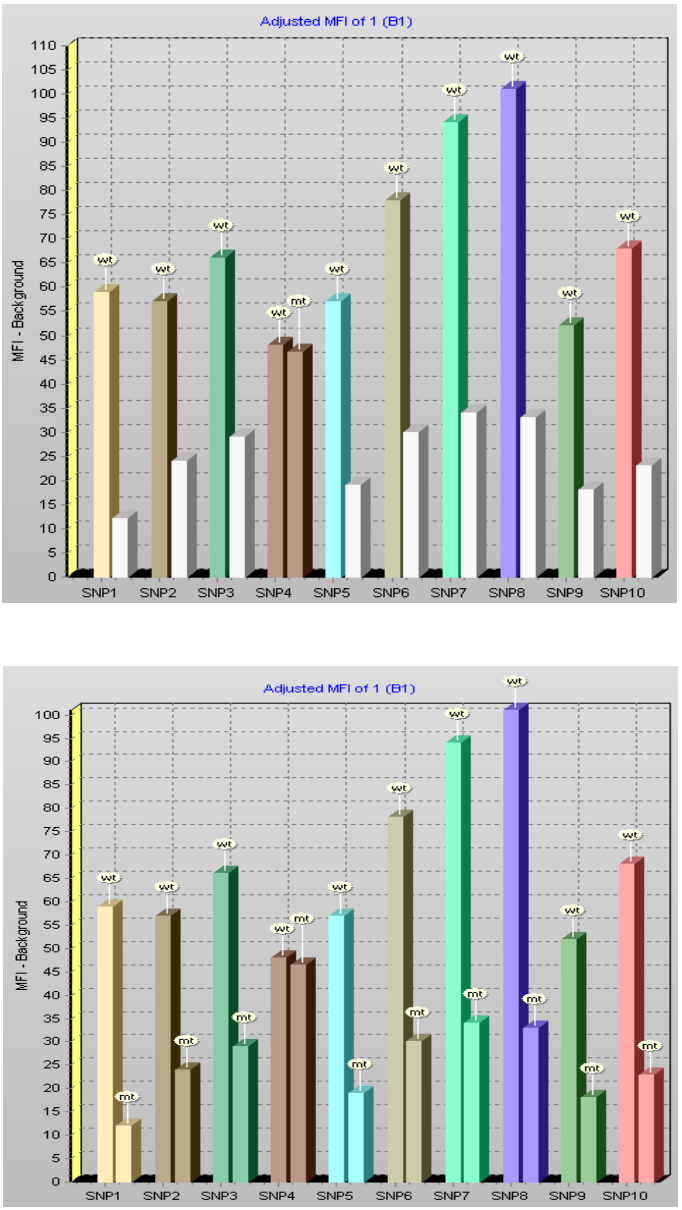


Figure B.9 Multi Compare graph, only called alleles painted (top), all alleles painted (bottom)

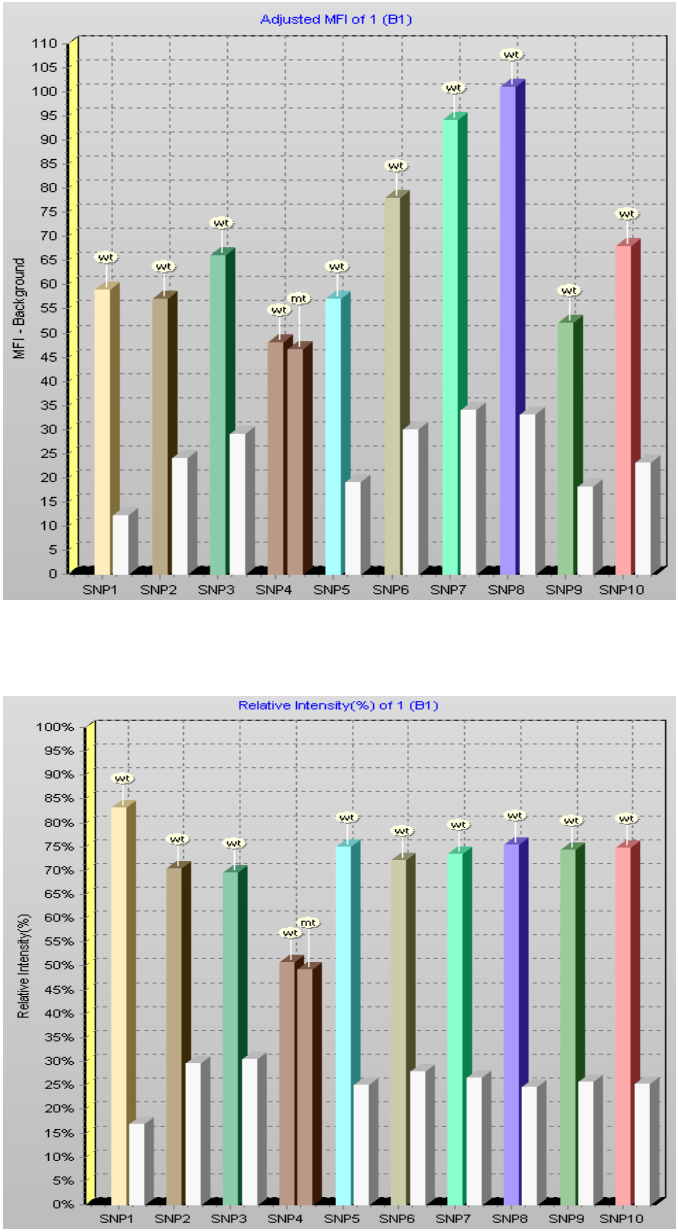


Figure B.10 Multi Compare graph, background-adjusted MFI data (top), percent relative intensity data (bottom)

B.4
Allele Call View

In the Allele Call View tab, you can specify defaults for the Allele Call table.

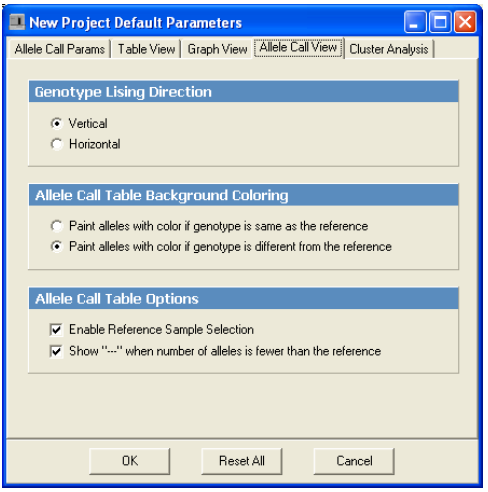


Figure B.11 New Project Default Parameters, Allele Call View tab

Genotype Listing Direction

Vertical Choose this option to display genotype calls in a vertical list in the Allele Call table (Figure B.12).

Horizontal Choose this option to display genotype calls in a horizontal list in the Allele Call table (Figure B.12).

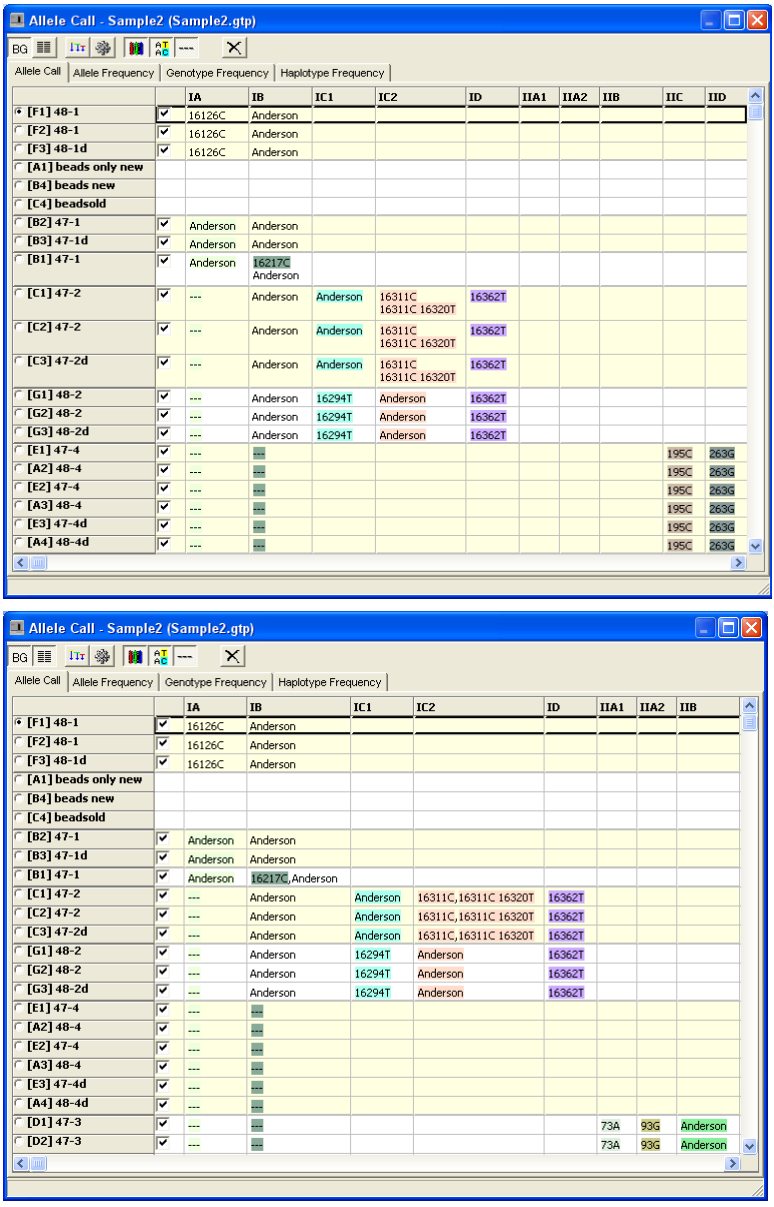
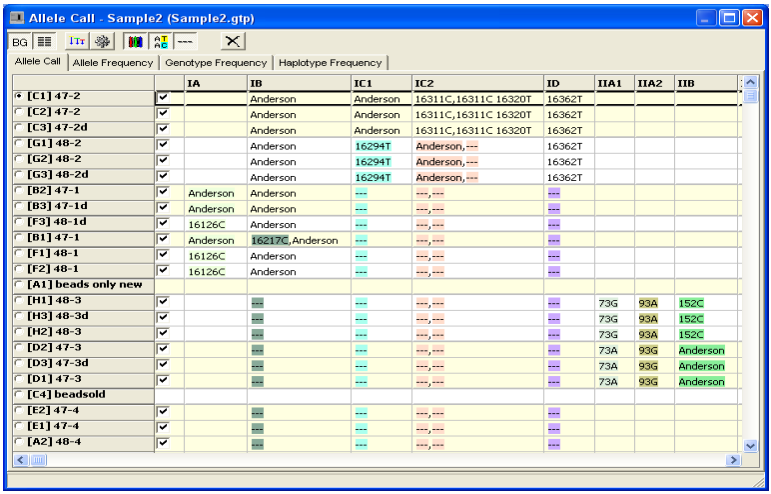


Figure B.12 Allele Call table
Vertical allele name list (top), horizontal allele name list (bottom)

Allele Call Table Background Coloring

You can sort the Allele Call table by expression level (MFI data) or haplotype to a user-selected reference sample. The Allele call table displays the reference sample in the top row of the table.

Paint alleles with color if genotype is same as the reference	Choose this option to paint (highlight) alleles in the Allele Call table that have the same genotype as the user-selected reference sample (Figure B.13). The group or allele color is applied (depending on what was specified in the Parameter Setting dialog box).
Paint alleles with color if genotype is different from the reference	Choose this option to color alleles in the Allele Call table that is do not have the same genotype as the user-selected reference sample (Figure B.13). The group or allele color is applied (depending on what was specified in the Parameter Setting dialog box).



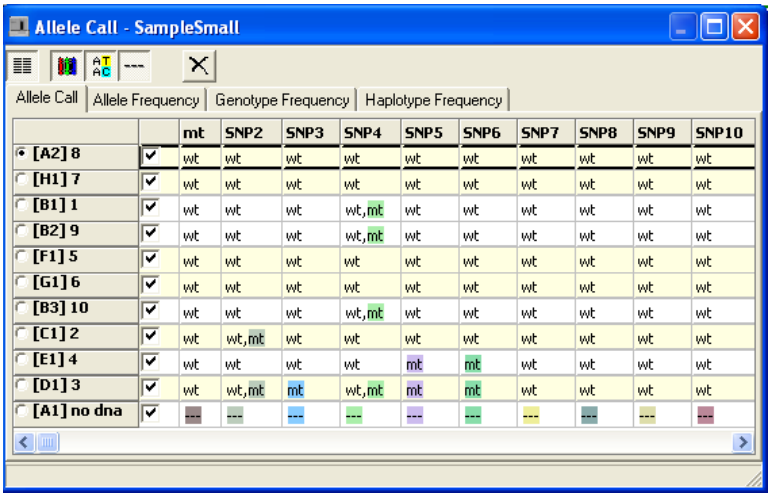


Figure B.13 Allele Call table
Same genotypes painted (top), different genotypes painted (bottom)

Allele Call Table Options

Enable reference sample selection — Choose this option to display the reference sample selection radio buttons in the Allele Call table (Figure B.14).

Show "---" when number of alleles is fewer than the reference — Choose this option to help identify samples that have fewer called alleles than the reference sample (Figure B.14).

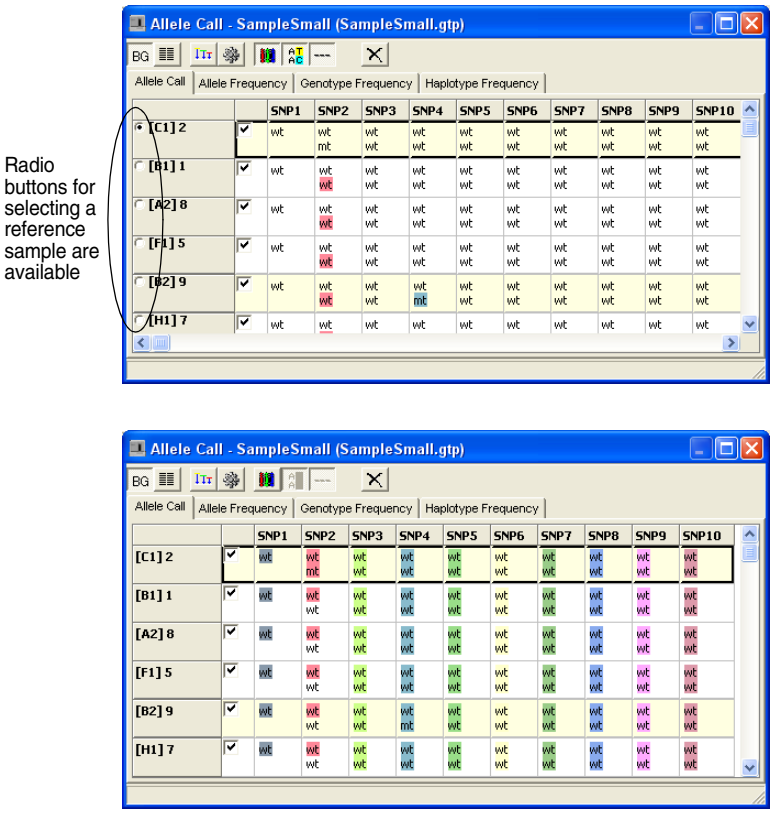


Figure B.14 Allele call table, reference sample selection enabled (top), disabled (bottom)

B.5
Cluster Analysis

Default Cluster Analysis Method	Make a selection from this drop-down list to set the default cluster analysis tool.
Cluster Analysis By Expression	Choose this option to cluster samples according to the MFI data.
Cluster Analysis By Genotype/Haplotype	Choose this option to cluster samples according to the genotype of all the alleles.

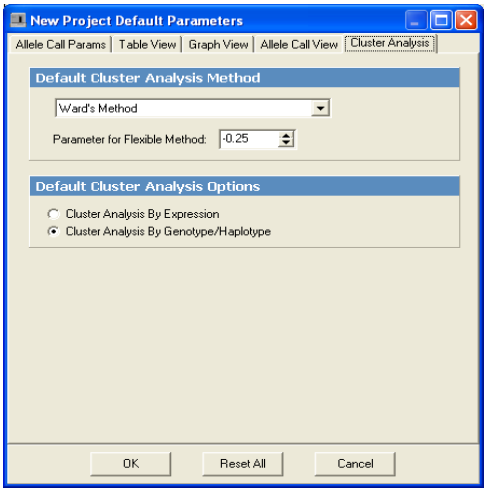


Figure B.15 New Project Default Parameters, Cluster Analysis tab

B.6
Resetting the Default Parameters

To reset the options and parameters to the default factory settings, click **Reset All** (Figure B.15). At the prompt, click **OK**.

C.1 Main Toolbar

Figure C.1 Main toolbar

Table C.1 Main toolbar buttons and functions

Menu Bar Command	Main Toolbar Button	Function
File → Open CSV File		Displays the Open dialog box so that a Luminex® results file (.csv) may be opened.
File → Open Project File		Displays the Open dialog box so that a project (.gtp) may be opened.
File → Save Project		Displays the Save As dialog box so that a project (.gtp) may be saved.
		Displays the Typing table for the active results (.csv or .gtp).
		Displays the Multi Graph view for the active results.
		Opens a window that displays the Homology table and chart for the active results.
Function → Allele Call		Displays the Allele Call table for the active results.
		Opens the Report Manager.
Window → Cascade		Tiles the Typing table and Multi Graph views for the active results in a cascade.
Window → Tile Horizontally		Tiles the Typing table and Multi Graph views for the active results horizontally.
Window → Tile Vertically		Tiles the Typing table and Multi Graph views for the active results vertically.

C.2
Typing Table Toolbar

Figure C.2 Typing table toolbar

Table C.2 Typing table toolbar buttons and functions

Typing Table Toolbar Button	Function
	Opens the Parameter Setting dialog box for the active results (.csv or .gtp).
	Displays the relative intensity (r) of each allele in a group.
	Displays the background-adjusted MFI data for the alleles in the Typing table.
	Displays the bead count data in the Typing table.
	Displays the allele rows of the first locus (group) in the Typing table with a blue background. Displays the allele rows of the next locus with a white background. This alternating use of color distinguishes the allele members of each group and gives the table background a *striped* appearance.
	Uses a color gradient to indicate the relative expression level of the alleles called at each locus in a sample (a lighter shade represents a lower expression level). Alternating colors (defaults are blue and red) distinguish the alleles (rows) of the loci (groups) in the table.
	Displays the dendrogram.
	Displays the Multi Graph view for the active results.
	Sorts the samples in the Typing table by homology to the expression levels of a user-selected sample.

Table C.2 Typing table toolbar buttons and functions

Typing Table Toolbar Button	Function
	Resets the samples in the Typing table to the default order (the order that sample data were acquired in the Luminex® system).
	Show the Typing table with a separate tab for each locus.
	Displays a Type column for each locus in the Typing table.

C.3 Multi Graph View Toolbar

Figure C.3 Multi Graph view toolbar

Table C.3 Multi Graph view toolbar buttons and functions

Multi Graph View Toolbar Button	Function
	Opens the Parameter Setting dialog box for the active results (.csv or .gtp).
	Displays 3-dimensional bars in the Multi Compare graph.
	Displays allele name tags in the Multi Compare and Depth bar graphs.
	Displays a legend of allele names for the Multi Compare and Depth bar graphs.
	Displays the bars in the Multi Compare and Depth bar graphs with a color gradient.
	Displays the MFI and RI thresholds in the Multi Compare bar graphs.
	Paints only the called alleles in the Multi Compare and Depth bar graphs.
	Displays the x-axis labels vertically in the Multi Compare graph, Depth graph, and Threshold editing tab.

Table C.3 Multi Graph view toolbar buttons and functions

Multi Graph View Toolbar Button	Function
	Includes the group and allele name in the x-axis label of the Multi Compare graph, Depth graph, and Threshold editing tab.
	Displays a subset of the x-axis labels so that none of the labels overlap in the Multi Compare graph, Depth graph, and Threshold editing tab.
	Plots the background-adjusted MFI data in the Multi Compare and Depth bar graphs.
	Plots the percent relative intensity data in the Multi Compare and Depth bar graphs.
	Puts the Multi Graph view in *Two Sample Comparison* mode that enables you to plot a Sample by Sample scatter graph.
	Hides or unhides the Heat map in the Multi Graph view.
	Creates a dendrogram of the samples in the active results and displays the Clustering Tool dialog box.
	Displays the Typing table.
	Use the slider to adjust the display angle for the name tags in the Multi Compare graph.
	Displays a drop-down list of size options for the horizontal dimension of the bars in the Multi Compare and Depth graphs.
	Displays a drop-down list of size options for the vertical dimension of the bars in the Multi Compare and Depth graphs.

A

B

C

D

E

G

H

I

L

M

N

O

P

R

S

T