

Version 2.0 for Microsoft[®] Windows

MasterPlex™ GT 2.0 Genotype Analysis Software

Mirai<mark>Bio</mark>

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For Research Use Only

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MasterPlex™ GT 2.0

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

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CHAPTER 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 \Rightarrow 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 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).

InstallShield Wizard		×
	Welcome to the InstallShield Wizard for MasterPlex GT The InstallShield® Wizard will install MasterPlex GT on your computer. To continue, click Next.	
	< Back Cancel	

Figure 2.1 InstallShield Wizard

2. To continue the installation, click Next. \Rightarrow 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.
 - \Rightarrow The License Information dialog box appears (Figure 2.4).

License Information		×
Product Name:	MasterPlex GT version 2.0.0 Build 185	
User Name: Institute: Division:		
Number of Licenses: Date Issued: Licensed Version:	:	
Licensed Machine ID: Expiration Date:		
Machine ID of this PC:	2E71-62B3-30BE	
Install New License	Detain New Licenses Demo Licenses Demo Licenses	

Figure 2.4 License Information dialog box

- 2. To view instructions on how to obtain a license (.lic), click Obtain New Licenses.
- 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.
 - \Rightarrow The license is installed.
- To view the license information, select Help → View License Info from the menu bar.
 - \Rightarrow The license information is displayed (Figure 2.5).

License Information		×
Product Name:	MasterPlex GT version 2.0.0 Build 185	
User Name:	Katherine Shigekawa	
Institute:	Cogent Comm	
Division:	-	
Number of Licenses:	1	
Date Issued:	2003/10/29	
Licensed Version:	version 2.0.0 Build 0	
Licensed Machine ID:	4610-59B8-7B5E	
Expiration Date:	2003/12/28	
Machine ID of this PC:	4610-59B8-7B5E	
Install New License	Obtain New Licenses Demo Licenses Oose	

Figure 2.5 License information

CHAPTER BEFORE YOU BEGIN

This chapter explains the MasterPlex \mathbb{M} 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.

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 3.1	MasterPlex™	GΤ	bead	naming	conventions
-----------	-------------	----	------	--------	-------------

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

	names at each locus.												
				Λ									
			/	/									
Typing	- Sample2 (Sam	ple2.gtp)		/								- 5] ×
%	💗 🕎 🔳	🔁 🖹	24	õ									
			Locus	IA.				в				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	-	-	-	-	-	-	-	-	-	
<u>B2</u>	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	
<u>C3</u>	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	1
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	~

First row shows locus or group name (the prefix set in the Luminex software). Second row shows allele names at each locus.

Figure 3.1 Typing table

Bead names sorted by prefix and allele name.

%	¥ 🛛 📖	= > <u>1</u>	AJ Ю	õ									
			Locus		1				1				~
			Beads->	IA 161240	IA 161260	IA 16129/	IA Anders	IB 162170	IB 16223T	B 162240	B Anders	IC1 16292	
v/ell 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	1
C2	47-2	6409	Sample Empty	2	2	2	2	74	39	65	[IB]MFI=7:	1, r=17.9%	6
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	
н	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** \rightarrow **Set Application Options** from the menu bar.

 \Rightarrow The Application Options dialog box opens (Figure 3.3).

Application Options
General Background Clustering Tool Plugins
Bead Name Style
C Locus Name + Allele Name C Original Bead Name
Start Up Window After Data Loading
Show Table View C Show Graph View
Table View Gradation Background
Use Allele Call Color for gradation background
Group Color #1 Change Color
Group Color #2 Change Color
Heatmap Options
Heatmap Bar Size: 3 Pixel(s) / Allele
OK Reset All Cancel

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:

Click the Parameter Setting button ⁽¹⁾

 \Rightarrow The Parameter Setting dialog box appears (Figure 3.4).

Parameter Setting									
Group s	et: Save	Cancel	ок						
I Para I Use	ameter set group col	up for the indiv or for Chart an	idual bead. d Allele Call Tabl	Minimum Events: e	20 count for ea	ch bead Lookup Table			
Prefix	Group Na	ame Type	Lookup Table	Allele Name	%Reportable Level	Intensity Threshold	Call Intensity		
IA	IA	Other							
				16124C	25.0%	35			
				16129C	25.0%	35			
				Anderson	25.0%	35			
IB	IB	Other		400470	25.000	25			
				16217C 16223T	25.0%	35			
				16224C	25.0%	35			
754	104	0.11		Anderson	25.0%	35			
101	101	Other		16292T 16295T	25.0%	35			
				16294T	25.0%	35	~		
- Group//	Allele Identi	ifier							
Group	Prefix: IA	# of	beads in this gr	oup: 4			** Edit Bead Names		
Group	Name: 🔝			Chang	e Color				
	Ploidy: 💿	Diploid C	Haploid C O	ther Apply	his Ploidy to all groups	(loci)			
Allele N	lame: An	iderson		Chang	e Color Apply	to all alleles in the order in each group.			
					Apply	to all same name allele:			
-Allele C	all Paramet	ters for IA And	erson						
⊙ Us	e Relative I	Intensity for All	ele call						
	Denest		50 00 -00-00						
	Reports	able Level: 14	3.0 % of total	ntensity					
	Intensit	y Threshold: 3	5 (MFI)						
C Inte	ensity base	ed Allele Call							
	Call any	ything bigger th	an 50 M	1Fi as an Allele					
			A	pply to all beads					

Figure 3.4 Parameter Setting dialog box

2. Click Edit Bead Names.

 \Rightarrow The Edit Bead Names dialog box opens (Figure 3.5).

Edit Bead Names	×
[A 16124C [A 16128C [A 16128C [A Aderson [B 16217C [B 16217C [B 16223T [B 16224C [B Anderson [C1 162921 16295T [C1 162941 16296T [C1 16394C [C1 163946 [C2 16396] [C2 16396] [C2 16396] [C2 163101 [C2 163102 [C2 163104] [C2 163207] [C2 2405307] [C2 2405307] [D1 16362C [D 16362C [D1 16362C [IA1 736 [IA2 936	
OK Cancel	

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

🔳 MasterPl	ex GT - [Typing - S	ample2 (Sampl	e2.gtp)]									
File Edit	View Function Optic	on Window Help							-	>		
* * * * * * * * * * * * * * * * * * *												
	Locus IA IB											
			Beads->	16124C	rernen	464004	Anderson	16217C	16223T			
Vell Name	Sample Name	Total Events	Notes		Edit Bead Na	me						
F1	48-1	5162	Sample Empty	1	94	0	10	141	61			
F2	48-1	5286	Sample Empty	1	88	1	12	142	61			
F3	48-1d	6305	Sample Empty	1	86	1	10	130	65			
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			
B3	47-1d	6894	Sample Empty	5	6	3	51	158	74			
B1	47-1	5519	Sample Empty	4	5	4	58	197	75			
C1	47-2	6618	Sample Empty	0	2	1	2	75	47			
C2	47-2	6409	Sample Empty	2	2	2	2	74	39			
C3	47-2d	8115	Sample Empty	1	2	2	2	69	45			
ମ	48-2	5122	Sample Empty	0	3	1	1	60	44			
G2	48-2	5135	Sample Empty	1	4	2	3	59	45			
G3	48-2d	5012	Sample Empty	0	1	1	2	48	38			
E1	47-4	5063	Sample Empty	1	0	2	1	1	1			
1.0	1	1	1	1 .	1	1	2	r ,	1			

Figure 3.6 Typing table

Right-click a bead name to edit the name.

Change Bead Name									
New Bead Name									
AmetX									
OK Cancel									

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.

	-											
	Group set	Save setting	gas li	mport Setting]	Cancel	ок					
	I → Parameter setup for the individual bead. Minimum Events: 20 count for each bead I → Use group color for Chart and Allele Call Table Lookup Table Lookup Table											
	Prefix G	roup Name	Туре	Lookup Table	Allele Name	%Report	Intensity Call Inten. 🔨					
Allele name 16217C	IA I/	Y	Diploid		16124C 16126C	25.0% 25.0%	35 35					
Selected	IB IE	9	Other		16129A Anderson	25.0% 25.0%	35 35					
					16223T 16224C Anderson	25.0% 25.0% 25.0% 25.0%	35 35 35 35					
	IC1 K	21	Other		40000T 4000ET	ne nø/	× ×					
To edit a	-Group/Allele Identifier Group Prefix: ^{1B} # of beads in this group: 4 ** Edit Bead Names											
group name,	Group Na	ime: IB			Change Color							
name (above)	Pic	oidy: C Diploi	d CH	laploid 💿 Other	Apply this Ploid	ly to all group:	to ell elleles in the					
name here.	Allele Nar	ne: 16217C			Change Color	same	order in each group. to all same name alleles					
	Allele Call	Parameters fo	or IB 16217	c								
To edit an allele	O Use I	Relative Intens	ity for Allel	e call								
name, select an	Reportable Level: 25.0 % of total intensity											
allele name	Intensity Threshold: 35 (MFI)											
(above) and edit	C Intensity based Allele Call											
the name here.		Call anything bigger than 50 MFI as an Allele										
				Apply1	to all beads							

Figure 3.8 Parameter Setting dialog box

- 2. Edit the name in the Group Name or Allele Name box.
- 3. Click Apply.

 \Rightarrow 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.

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.

Table 3.3 MasterPlex GT table and graph formats

🔳 Pa	ramete	er Setti	ng						
Group :	set: Sa	ve setting	gas li	Cancel		ок			
∏ Par ∏ Usr	rameter : e group	setup for color for	the individ Chart and	count for	each be	ad Lookup Table			
Prefix	Group	Name	Туре	Lookup Ta	able	Allele Name	%Report.	Intens	sity Call Inten. 📩
SNP1	SNP1		Diploid				25.0%	35	
SNP2	SNP2		Diploid			wt mt wt	25.0%	35	
SND3	SND3		Diploid			mt	25.0%	35	
SITE 3	SINFO		Dipiola			wt	23.076	33	
SNP4	SNP4		Diploid			mt	25.0%	35	
5/11 4	ON T		Dipiola			wt	20.070	00	~
<						und .			
Group	Allele Id	entifier							
Group	Prefix:	SNP1	# of b	eads in this	s group: 2	2		** E	Edit Bead Names
Group	Name:	SNP1		_		Change Cold	ar I		
oreap	Ploidy:	 Diploi 	d C H	laploid (Other	Apply this Plo	bidy to all group	os (loci)	
Allele I	Name:					Change Cold	or Appl	y to all a e order i y to all a	alleles in the in each group. same name alleles
-Allele (Call Para	meters fo	or SNP1						
🖲 Us	se Relati	ve Intens	ity for Allel	e call					100
	Rep	ortable L	evel: 25.	0 % of to	tal intens	sitv			100
	Inter	nsity Thre	eshold: 35	(M	IFI)	,			80
C Int	ensity b	ased Alle	ele Call bigger the	50	MELoo	an Allele			40
	Call	anything	bigger that	190	wrtas	an Allele			20
				A	pply to al	ll groups (loci)			MFI Allele Ratio

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

MasterPlex GT - [Typing - San	npleSmall (S	SampleSmall.	gtp)]										×
Ele Edit Yew Function Option Window Help													
×			1	Locus	SNP1		SNP2		SNP3		SNP4		1
🗅 Sample2				Beads->	wt	mt	wt	rnt	wt	rnt	wt	rnt	
	Well Name	Sample Name	Total Events	Notes									
🔣 📶 Multi Graph	F1	5	2860		354	21	475	39	656	48	576	40	
SampleBase	G1	6	2711		307	23	702	40	1244	30	706	71	
- Typing Table	H1 en	0	2517	-	614	A2	593	81	1080	48	822	60	
Multi Graph	C1	2	2652		605	44	725	645	368	68	442	70	
SampleSmall	B1	1	2582		50	0	50	8	45	21	36	33	
Typing Table	82	9	2736		450	33	567	37	700	80	370	273	
Multi Graph	B3	10	3100		334	31	579	47	967	114	783	574	
	D1	3	2924		346	34	616	661	20	981	527	614	
	E1	4	3103		486	35	607	41	932	80	583	85	
MFI Adjusted MFI Count	A1	no dha	21		-	-	-	-	-	-	-	-	
Bead Ave. SD CV% SNP1 wt 492 151.2 30.7% SNP1 mt 50 6.9 13.8% SNP2 wt 649 65.0 13.1% SNP2 mt 246.0 12.2%			Select sa	mples	s (row	/s) to	sho	w sta	tistic	s.			
SNP3 wt 922 380.3 41.2% SNP3 mt 109 64.8 59.3% SNP4 wt 693 165 24.0% 24.0%													
													-
Project Manager			Typing	table	in th	e Pro	ject V	Wind	ow.				

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

Figure 3.10 Project Manager and Project Window

Typing table displayed in the Project Window.

🛄 MasterPlex GT - [Graph - Sam	ɪpleSmall]	- 7 🛛
He Edit View Option Window	Heb	_ 8 ×
SampleSmall	🖉 🏶 🗃 🗞 🔚 💻 🕼 👰 👼 🎼 🌾 🦉 🎼 🎼 🔛 🔣 🖿 Hx1.0 Vx1.0	
Typing Table	Pos Sample Name 📕 👔 Multi Compare 🖫 Depth 👫 Threshold Editing 🐖 Sample by Sample by Sample (Allele by Allele
Multi Graph	A1 no dha	
🗅 SampleBase	B1 1 Adjusted MF1of1 (B1)	[SNP1]
Typing Table		at 59
🗆 📶 Multi Graph	D1 3 g 1,500	[SNP2]
C Sample1		at 57
Typing Table	F1 5	[SNP3]
Multi Graph	La1 6	at 66
L Sample2		[SNP4]
Typing Table		ut 48 mt 47
- 📶 Multi Graph	P3 10 SNP1 SNP2 SNP3 SNP4 SNP5 SNP6 SNP7 SNP8 SNP9 SNP10	
	Adjusted MFI of 4 (E1)	[SNP1] ut 405
MFI Adjusted MFI Count		mt 47 [SNP2]
Bead Ave. SD DV% 🔺	ີ່ ສີ1,000 - 🙀 🖤 🚛 🛄 🛶 ໜັ	mt 67 [SNP3]
SNP1 wt 392 295.6 75.4%		ut 953
SNP1 mt 38 22.5 59.7%	· ਦੁ ***	[SNP4]
SNP2 wt 424 517.6 75.0%		ut 595
SNP3 wt 707 559 7 79 2%	SNP1 SNP2 SNP3 SNP4 SNP5 SNP6 SNP7 SNP8 SNP9SNP10	
SNP3 mt 58 29.5 51.2%	Adjusted MELot 8 (A2) 98, 94	
SNP4 wt 492 402.9 81.8%		[SNP1]
SNP4 mt 73 26.3 35.9%	2 1,500 WV WV	mt 54
SNP5 mt 297 449.8 151 52		ut 600
SNP6 wt 372 535.4 143.9%		mt 97 [SNP3]
SNP6 mt 296 418.4 141.5%		ut 1101
SNP7 wt 1085 871.2 80.3%		[SNP4]
SNP7 mt 53 16.9 31.9%		ut 834 mt 74
SNP8 wt 1032 818.9 79.4%	- SNP1 SNP2 SNP3 SNP4 SNP5 SNP6 SNP7 SNP8 SNP9 SNP10 -	
SNP3 mt 124 36.4 77.7%		F I
	Max: 1731 (AUTO)	
· · ·		
Project Manager	Project Window displaying	
includes file tree	(ton) the Leet man and Multi	
includes life tree	נוסף) נופ הפגו המף מוט אונונו	
and Statistics tab		
	Compare graph	

Figure 3.11 Project Manager and Project Window

Multi Compare graph in the Project Window.



Figure 3.12 Heat map

	Haplotype for sample 47-1d Row of locus												
Allele Call - Sample2.gtp)													
BG 🎫 📭 🛞	AT AC	- x			/			/					
Allele Call Allele Frequency	Ge	notype Frequ	iency Haple	otype Freque	hcy			/					
		IA	IB	IC1 /	IC2	ID	IIA1 [′]	IIA2	IIB	IIC	IID	_	
@ [B2] 47-1		Anderson Anderson	Anderson										
○ [B3] 47-1d		Anderson Anderson	Anderson	\supset									
○ [B1] 47-1	~	Anderson Anderson	16217C Anderson										
⊂ [F3] 48-1d	•	16126C 16126C	Anderson										
C [F1] 48-1	•	16126C 16126C	Anderson										
⊂ [F2] 48-1	•	16126C 16126C	Anderson										
⊂ [G3] 48-2d	~		Anderson	16294T	Anderson	16362T							
⊂ [G2] 48-2	~		Anderson	16294T	Anderson	16362T							
C [G1] 48-2	~		Anderson	16294T	Anderson	16362T							
⊂ [C3] 47-2d	~		Anderson	Anderson	16311C 16311C 16320T	16362T							
C [C2] 47-2	•		Anderson	Anderson	16311C 16311C 16320T	16362T							
C [C1] 47-2	~		Anderson	Anderson	16311C 16311C 16320T	16362T						~	
1													
Sample name column													

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

🔳 Homo	Homology Plot - Sample2													×
Homology Table Homology Chart														
		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	0.98959	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 3.14 Homology table

Correlation coefficient between sample genotypes are displayed.





CHAPTER 3 Before You Begin



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.*
Pos	Sample Name	
F1	5	
B2	9	—— <u>h</u>
B3	10	h
G1	6	<u>_</u> _
H1	7	
A2	8	
C1	2	
E1	4	
D1	3	
A1	no dna	1
B1	1	J

Figure 3.20 Dendrogram

Example cluster analysis results.

CHAPTER GETTING STARTED Working with Luminex® 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	Displays the Typing table or Multi Graph view of
(Figure 4.2):	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.

CHAPTER 4 GETTING STARTED

D San d r San D San C San	nple2 Typin Multi npleE Typin Multi Multi Multi	g Tabli Graph Base g Tabli Graph Graph Graph	e	X	Project tree
MFI	Adju	sted MF	I Cou	int	
Bea	d	Ave.	SD	CV% 🔨	
IA 1612	4C	6	0.6	10.2%	
IA 1612	6C	6	0.5	9.1%	
IA 1612	9A	4	0.6	15.7%	
IA Ande	rson	55	3.5	6.4%	Statistics table
IB 1621	7C	170	24.1	14.1%	
IB 1622	3T	73	5.3	7.3%	
IB 1622-	4C	148	16.2	11.0%	
IB Ande	rson	308	3.8	1.2%	
IC1 162	92T 1	4	1.5	41.7% 💙	
<				>	

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.

📕 Typing - Sample2 (Sample2.gtp)] X			
%	è % ● 🖺 📰 📑 ≥ 🛄 🚑 ∽ 🗰 🎯												
			Locus	IA				в				IC1	^
			Beads->	16124C	16126C	16129A	Anderson	16217C	16223T	16224C	Anderson	16292T 1	
Well Name	Sample Name	Total Events	Notes										Г
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	v

Figure 4.2 Project Window, Typing table view

	💶 MasterPlex GT - [Typing - Sample2 (Sample2.gtp)]														
III F	Ele Edit Vew Eurotion Onlino Window Help														
	ac Luc	1000	i uno			Window Golp								-	u ~
	🗅 Sample1 🛛 🖓 🔊 🛡 🔛 📖 💴 🖳 💱 🛄 🔍														
-	📄 Typin	g Table							1	L.				l	1 100
	📕 Multi	Graph						_	Locus	A				IB	
D :	Sample2								Beads->	16124C	16126C	16129A	Anderson	16217C	162
1	Typin	a Table	2			vvell Name	Sample Name	Total Events	Notes	5	5		55	152	_
	Multi	Granh				82	47-1	7615	Sample Empty	ļ	6		51	158	
ln a	SamalaF	Baca				B3	47-10	6640	Sample Empty	- Å	5	Å	58	197	
	Tunin	a Tobl				52	47-1	6305	Sample Empty	1	86	1	10	130	
	i ypin	g rable				F3	40-10	6160	Sample Empty	1	94	0	10	141	
]		Graph				F2	40-1	5786	Sample Empty	1	88	1	12	142	
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	📒 Typin	g Table	9			62	48.2	5135	Sample Empty	1	4	2	3	59	
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MFI	Adju	sted MFI	Cou	Int		C4	beadsold	951	Sample Empty		-	-	-	-	
	Bead	Ave	SD	CV96	T	H3	48-3d	4974	Sample Empty	1	2	1	2	4	
10.1	6124C	6	0.6	10.2%		H1	48-3	6188	Sample Empty	2	2	1	3	2	
10.1	61260	6	0.5	0.1%	"	H2	48-3	5242	Sample Empty	1	2	1	2	3	
	61200	4	0.0	15 7%		D2	47-3	6637	Sample Empty	1	1	2	1	4	
	orizon .		2.0	E 490		D3	47-3d	6952	Sample Empty	0	2	2	1	4	
04.4	00470	470	0.0	44.40		D1	47-3	4359	Sample Empty	1	2	-1	3	3	
01	0217C	170	24.1	14.1%		E1	47-4	5063	Sample Empty	1	0	2	1	1	
81	62231	73	5.3	7.5%		A2	48-4	7017	Sample Empty	2	1	1	2	1	
181	6224C	148	16.2	11.0%		E3	47-4d	6462	Sample Empty	2	1	2	1	2	
BA	nderson	308	3.8	1.2%		E2	47-4	3109	Sample Empty	0	0	2	1	1	
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101	16294T	4	0.0	0.0%		<									>
101	16294T 1	4	0.6	13.3%											
-		_	_	_											

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
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 <mark>Ⅲ</mark> 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

Group (locus) names (first row) and allele names (second row)

Project Window toolbar

Typing	Typing - Sample2 (Sample2.gtp)												
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			Locus	IA				B				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	-	-	-	-	-	-	-	-	-	
84	beads new	7787	Sample Empty	-	-	-	-	-	-	-	-	-	
C4	beadsold	951	Sample Empty	-			-	-	-	-	-	-	
82	47-1	7615	Sample Empty	5	5	4	55	153	65	135	304	2	
83	47-1d	6894	Sample Empty	5	6	3	51	158	74	140	300	3	
81	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	~
1	1	1	1										
Well	Sample	Total beac	ls Notes	enter	ed in	the							

location name counted per Luminex® software sample





Figure 4.5 Project Window

Multi Graph view shows the Multi Compare graphs for the selected samples.

Viewing Project Information

- 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).
- Project Information 🗅 Sample1 << CVProgram FilesVHtachiSoftWasterPlex GT/SampleData/SampleBase.csv >> Program: LUMINEX Build: VERSION 1.7.69 Typing Table 📶 Multi Graph 5/29/02 1:23:45 PM Date: Date: 5/29/02 1:23:45 Serial Number: LX10 Session: 0000-00-00001 Operator: SAMPLE Sample2 LX10000000000 Typing Table Heater Temparature: Number Of Samples: Minimum Events: 20 📙 Multi Graph 25 Typing T 📶 Multi Gra Delete SampleSma 📗 Typing T Merge All 👖 Multi Gra Project Merge Wizard Copy Info Close

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

 \Rightarrow The Open dialog box appears (Figure 4.7).

Click the Open CSV File button . Alternatively, select File → Open CSV File from the menu bar.

Open **?**× 🔻 듣 🖆 🎫 Look in: 🗀 SampleData Sample2.csv SampleBase.csv SampleSmall.csv HLA-A_B_DR_BLD.csv LayerSampleA.csv LayerSampleC.csv LayerSampleG.csv LayerSampleT.csv Sample1.csv File name: Open Files of type: Luminex Data file (*.csv, *.mdb) • Cancel

Figure 4.7 Open dialog box

2. Double-click the .csv file that you want to open.

 \Rightarrow 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).



If the field yew Function Cotion Window Heb Image: Control of the second s	MasterPlex GT - [Typing - Sample2 (Sample2.gtp)]										
Sample1 No No <t< td=""><td colspan="9">Ele Edit Yew Function Qotion Window Help - 🗗 🗙</td></t<>	Ele Edit Yew Function Qotion Window Help - 🗗 🗙										
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Ist 16294T 4 0.00 0.0% Ist 16294T 4 0.6 13.3% Project Manager Project Window displaying the Typing table	IC1 16292T 1 4 1.5 41.7%	A4	48-4d	6426	Sample Empty	1	1	2	2	2	~
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includes file tree (top)	includes file tree (tor))									
and Statistics table	and Statistics table	-,									
	and Statistics table										
	(bottom)										
	(bottom)										

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.

 \Rightarrow 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.
 - \Rightarrow 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).

 \Rightarrow The Project Merge Wizard appears (Figure 4.13).



Figure 4.12 Project Manager

CHAPTER 4 GETTING STARTED

Project Merge Wiza	ırd 🛛 🔀
	Sample Merge Merge Some projects with the same bead set.
#	Layer Merge Merge Some projects with the same Wells.
	Close

Figure 4.13 Project Merge Wizard

- Click the Sample Merge button ____.
 - \Rightarrow 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.

_		
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11		

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).

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

 \Rightarrow The Project Merge Wizard appears (Figure 4.16).



Figure 4.15 Project Manager

Project Merge Wiza	d	×
	Sample Marge Marge Some projects with the same bread set.	
	Layer Merge Merge Some projects with the same Wels.	
	Close	

Figure 4.16 Project Merge Wizard

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

Make a selection from the list of open projects	pen projects th oject selected project in the r	at can be layer merged with the above. If you do not want to include nerge, remove the check mark.
💷 Layer Merge Wizard 🛛 🖉		
Top Project	Locus - Beads	Project
LoverSampleA		~
	A	LayerSampleA
✓ LayerSampleC	C	LayerSampleC
🗹 LayerSampleG 🦰	G	LayerSampleG
LayerSampleT	ПТ	LayerSampleT
	Locus2	
	A	LayerSampleA
	C	LayerSampleC
	G	LayerSampleG
	ПТ	LayerSampleT
	E Locus3	
	A	LayerSampleA
	C	LayerSampleC
	G	LayerSampleG
	Т	LayerSampleT
	E Locus4	
	A	LayerSampleA
	C	LayerSampleC
	G	LayerSampleG
	IT	LayerSampleT
	E Locus5	
🔽 Delete Project for Merge	A	LayerSampleA
	C	LayerSampleC
OK Accept Cancel	G	LayerSampleG
		LayerSample I 🕥
Choose this option to	Bead list	Project names
remove all but the 'top	organizes	
project' name from the	names by	
Project Manager after	locus (arou	n)
the merce	name	۲ <i>۲)</i>
	numo	

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.



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).

I Layer Merge Wizard			
Top Project	Locus - Beads	Project	
Sample1	⊟ II' - 73A - 73G 934	Sample1 Sample1 Sample1	^
	036	Sample1	
	18	Edit Read Name	7
	161240	Sample1	
	16126C	Sample1	
	16129A	Sample1	
	Anderson	Sample1	
	III IB		
	16217C	Sample1	
	16223T	Sample1	
	16224C	Sample1	
	Anderson	Sample1	
	E IC1		
	16292T/16295T	Sample1	
	16294T	Sample1	
	16294T/16296T	Sample1	
	16298C	Sample1	
	16304C	Sample1	
	294T/296T/304C	Sample1	
	Anderson	Sample1	
	E IC2		
		Sample1	
	16311C	Sample1	
Delete Project for Merge.		Sample1	
OK L topont L Consol		Sample1	
Cancer	-16320T	Sample1	~
	Anderson .	Samnle1	×

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

Figure 4.19 Layer Merge wizard

LayerSampleA(Locus2 A)									
Bead Name : Locus2 A									
Suffix:									
☑ All Beads for this Project									
ок	Cancel								

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.

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^m 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:

Global NC = (Global NC₁ + Global NC₂ + ... 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:

Global NC = (Global NC_a + Global NC_b + 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.

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

Table 5.1 Computed background values

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.

 \Rightarrow 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).

* * * * * * * * * * * * * * * * * * *											
			Locus	SNP1		SNP2		SNP3		I	
			Beads->	wt	mt	wt	mt	wt	mt	Ι	
Well Name	Sample Name	Total Events	Notes								
F1	5	2860		354	21	475	39	656	48		
G1	6	2711		307	29	548	40	913	95		
H1	7	2517		409	35	702	106	1244	194	-	
A2	8	2802		614	42	593	81	1080	48		
C1	2	2652		605	44	725	645	368	68		
B1	1	2582		50	0	50	8	45	21		
B2	9	2736		450	33	567	37	700	80		
B3	10	3100		334	31	579	47	967	114		
D1	3	2924		346	34	616	661	20	981	1	
E1	4	3103		486	35	607	41	932	80		
A1	no dna	21		-	-	-	-	-	-		
		/	/								

Negative control

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).

 \Rightarrow 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.

 \Rightarrow 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 \rightarrow Set Application Options from the menu bar. \Rightarrow The Application Options dialog box opens (Figure 5.2).

💶 Application Options								
General Background Clustering Tool	Plugins							
Automatic Background Sam	nle Recognition							
Perform Automatic Background	d Sample Detection on data load							
Background Keywords								
bead only beads only	Add New Keyword							
bkg	Delete Selected Keyword							
blank								
NOTE: These parameters are effective only to new projects								
OK Re	eset All Cancel							

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).
 - \Rightarrow 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.
Add a Background Keyword Input a new keyword for background OK Cancel

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**.

6 ALLELE CALL PARAMETERS

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 .
 - \Rightarrow The Parameter Setting dialog box appears (Figure 6.1).

💶 Parameter Setting 📃 🗖 🔀											
Group set:	Save setting	gas Im	port Setting	•			Cancel	ок			
∏ Paramo ∏ Use gr	eter setup for oup color for	the individu Chart and A	al bead. N Ilele Call Table	4inimum Events	s: 20	count for ea	ach bead Lookup Table	•			
Prefix G	Froup Name	Туре	Lookup Table	Allele Name	%Reports	able Level 🛛 I	tensity Threshold	Call Intensity			
92R7 92	2R7	Other			25.0%	3:	5				
Amel A	mel	Other		T X	25.0%	3:	5				
DY51 D'	YS199	Other		Y 	25.0%	3:	5				
DY53 D'	YS391	Other		с т	25.0%	3:	5				
				c							
M9 M	9	Other		G 	25.0%	3:	5	~			
-Group/Alle	ele Identifier							í			
Group Pre	efix: 92R7	# of be	ads in this grou	ар: 2				** Edit Bead Names			
Group Na	me: 92R7			Cha	nge Color						
Ploi	idy: 🔿 Diploi	d CH	aploid 💿 Oth	er Appl	y this Ploidy	to all groups	(loci)				
Allele Narr	ne:		_	Cha	nge Color	Apply same of Apply	to all alleles in the order in each group to all same name all	eles			
Allele Call	Parameters f	or 92R7									
Use 5	elative Intens	ity for Allele	call					100			
	Renortable I	avel: 25.0	% of total int	laneitu				400			
	Internetty The	nahold: 35	(MED)	torioity				80			
	60 33.333										
C Intens	aty based Alle	ele Call						40			
	Call anything	bigger than	50 MF	'i as an Allele				20			
			Apply t	to all groups (lo	oci)			MFI Allele Ratio			
								11			

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	The software calls the allele if all of the following conditions are met:
Allele Call (see See "Relative Intensity Allele Call" on page 6.3)	 RI_{allele} ≥ user-specified RI threshold MFI_{allele} ≥ 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} ≥ a user-specified RI threshold (*reportable level*)
- $MFI_{allele} \ge a$ user-specified intensity threshold
- Click the button to display the Parameter Setting dialog box (Figure 6.2).

Para	motor Sotti	na									
	meter Setti	ng		_							
Group set	Cours antibio		west Catting	<u> </u>		Cancel	ок				
	Save setting	as in	ipon setting								
Param	neter setup for	the individu	albead. N	Ainimum Events	20 count for	each bead					
) Use g	roup color for	Chart and A	Allele Call Table	(·				
Prefix 1	Group Name	Туре	Lookup Table	Allele Name	%Reportable Level	Intensity Threshold	Call Intensity				
92R7 9	287	Uther		C	25.0%	35					
Amel A	Amel	Other		т	25.0%	35	-				
				X							
DYS1 D	YS199	Other		ř 	25.0%	35					
				ст							
DY53 D	YS391	Other			25.0%	35					
				G							
M9 N	19	Other			25.0%	35					
Group/All	ele identiner efiv: 92R7	# of be	ads in this grou	ıр: 2			** Edit Bead Names				
Group Na	ane: 9287		_		are Color						
Group Na	anie. jozini O ovi v			0.000	this Disidu to all area	ma (lasi)					
Plo	oidy: 🔘 Diploi	я сн	aploid 💽 Oth	er Appro	This Policy to all grou	ips (loci)					
Allele Na	me:			Char	nge Color	ly to all alleles in the e order in each group					
	,				App	ly to all same name al					
Allele Call	Parameters fo	or 92R7									
Use	Relative Intensi	ity for Allele	call				100				
	Reportable Le	evel: 25.0	% of total in	tensity			100				
Intensity Threshold: 35 (MFI) 80											
C Inten	60 33.333										
, incon	Call anything	higger than	50 MF	l as an Allele			20				
					. 1						
			Apply t	o all groups (lo	ci)		MFI Allele Ratio				

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 $MFI_a/(MFI_a+MFI_b) \times 100 \ge 25\%$.

25% = (33.333	3/(33.333+100))*100
Allele Call Parameters for 92R7	
Use Relative Intensity for Allele call	
Reportable Level: 25.0 % of total intensity	
Intensity Threshold 35.0 (MFI)	60 33.333
C Intensity based Allele Call	40 +
Call anything bigger than 50 MFI as an Allele	0 J

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.

 \Rightarrow 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 MasterPlexTM GT software calls an allele if the MFI is greater than a user-specified value.

- Click the Determinant 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.
 - \Rightarrow 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.)

- Click the button to display the Parameter Setting dialog box ((Figure 6.5)).
- 2. Click Edit Bead Names.

 \Rightarrow The Edit Bead Names dialog box opens (Figure 6.4).

Edit Bead Names 🛛 🔀
IA 16124C ▲ IA 16124C ▲ IA 16128C IA IA 16128A IA IA Afderson IB IB 16217C IB IB 16227T IE IB 162284 IA IB 16224C IB IB 16224T IE IC 16294T IC IC1 16294T IC IC2 16309G IC IC2 16309G IC IC2 16310C IC IC2 16310T IC IC2 Anderson ID ID 16362C ID IIA1 73G IIA2 IIA2 93G ¥
OK Cancel

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 is 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**.

🔳 Par	rameter	Setting								×	
Group s	set: Save	setting as	mport Setting	▼			Cancel		ок		
∏ Par ∏ Use	ameter set e group col										
Prefix	Group N	ame Type	Lookup Table	Allele Name	%Reporta	ible Level	Intensity Thresh	old C	all Intensity	^	
SNP1	SNP1	Diploid		wt mt	25.0%		35				
SNP2	SNP2	Diploid		wat .	25.0%		35 🔍				
5NP3	SNP3	Diploid		mt w/t	25.0%		35			-	 Group name SNP2 selected
SNP4	SNP4	Diploid		mt w/t mt	25.0%		35				
SNP5	SNP5	Diploid			25.0%		35			۷	
-Group/	Allele Ident	ifier	and to be to be					** Edit	Read Name	.	
Group	Prefix ON	PZ #010	ieaus in this grou	p. z		1			Dougrand	_	
Group	Name: SN	IP2	-	Ch	ange Color					_	
	Ploidy: 🖲	Diploid C H	Haploid C Oth	er App	oly this Ploidy	/to all grou	ps (loci)				Group Name
Allele 1	Name:			Ch	ange Color	App sam	ly to all alleles in t e order in each gr ly to all same nam	ihe roup. he allele	s		edit box
Allele C	all Parame	ters for SNP2								_	
🖲 Us	e Relative	Intensity for Allel	e call						1	00	
	Report	able Level: 25.	.0 % of total int	ensity				100	·		
	Intensit	y Threshold: 35	(MFI)					80	33.333		
C Int	ensity basi	ed Allele Call						60 41		I	
	Call an	ything bigger that	n 50 MF	as an Allele				20	j		
			Apply t	o all groups ((loci)			(N) L 1FI Allele Ra	tio	
										//.	

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

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

🖪 Parameter Setting	
Group set Cancel OK	
Parameter setup for the individual bead. Minimum Events: 20 count for each bead Use group color for Chart and Allele Call Table Lookup Table	
Prefix Group Name Type Lockup Table Allele Name %Reportable Level Intensity Threshold Call Intensity A SNP1 SNP1 Diploid	Group SNP2 selected
SNP2 SNP2 Dialoid	
SNP4 SNP4 Dipicid	 Group color swatch displays
SNPS SNP5 Dialoid 25.0% 35 💌	the color of the
Group/Allele Identifier Group Prefix: SNP2 # of beads in this group: 2 ** Edit Bead Names	selected group
Group Name: SNP2 Change Color	
Ploidy C Haploid C Haploid C Other Apply this Ploidy to all groups (loci)	
Allele Name: Change Color Apply to all alleles in the same order in each group.	
Allele Call Parameters for SNP2	
Use Relative Intensity for Allele call	
Reportable Level: 25.0 % of total intensity 100	
Intensity Interstroker 53 (MrI) 60 33.333 C Intensity based Allele Call Call anything bigger than 50 MFI as an Allele	
Apply to all groups (loci) MFI Allele Ratio	

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.
 - \Rightarrow The Group Name color swatch (or Allele Name color swatch) shows the selected color (Figure 6.7 and Figure 6.8).

🔳 Pa	ramet	er Setti	ing											X	
Group	set: Sa	ave setting	gas	Import Set	tting	•					Cancel		ок		
∏ Pa ∏ Us	rameter e group	setup for color for	the individ	lual bead. Allele Cal	. M I Table	finimum	Events	20	count for	each b	iead Lookup Tal	ble			
Prefix	Grou	p Name	Type	Lookup	Table	Allele I	Name	%Report	able Level	Intens	ity Threshold	d Cal	l Intensity	^	
SNP1	SNP1		Diploid	_			- 2	25.0%		35					
SNP2	SNP2		Diploid			wt mt 	- 1	25.0%		35				1111	
CND2	CMD2		Disloid			mt		15 NW		25					wt allele in
5115	SINFU		Dipiola			wt		23.0 %		35				_	group SNP4
CNID 4	CNID4		Distant			mt 🖌	-	05.000							selected
SNP4	SNP4		Dipiola			wt		25.0%		35					
						mt									
SNP5	SNP5		Diploid					25.0%		35					
Group	/Allele lo	lentifier										** Edit E	lead Name		
Group	Prefix:	SINP4	# 01 8	eads in tr	nis grou	р: 2					_	Luit L	cuunum	~	
Group	Name:	SNP4					Char	ige Color							
	Ploidy:	C Diploi	id O	Haploid	C Oth	er	Apply	this Ploid	ly to all grou	ips (loc	0				
Allele	Name:	wt		_			Char	nge Color		bly to all	alleles in the				
	rtaine.	1			~	<u> </u>		igo ooloi	san	le order bly to all	same name	up. alleles			
								_	<u> </u>						
										_		_			
												_	-		
															 Allele color
															swatch displays
															the color for the
															selected allele
														11.	

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.

 \Rightarrow 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.

 \Rightarrow 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.

 \Rightarrow 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.

 \Rightarrow The Create a New Group Set dialog box appears (Figure 6.11).

Create a New Group Set							
New Group Set Name:							
OK Cancel							

Figure 6.11 Create a New Group Set dialog box

3. Enter a name for the group set and click OK.

 \Rightarrow The group set (.xml) is saved and the name is added to the New Group Set name drop-down list (Figure 6.12).

Parameter Setting											
Group set	t: Group Set 1 Group Set 1 Group Set 2			•			Cancel	ок			
∏ Paran ▼ Use g	✓ Paramal Group Set 3 Jum Events: 30 count for each bead ✓ Use group color for Chart and Allele Call Table Lookup Table										
Prefix	Group Name	Туре	ookup Table	Allele Name	%Reporta	ble Level Ir	ntensity Threshol	d 🛛 Call Intensity 🔼			
SNP1 3	SNP1	Diploid			25.0%	35	5				
SNP2	SNP2	Diploid		vvt mt vvt	25.0%	35	5				
SNP3 S	SNP3	Diploid		mu 	25.0%	35	5				
SNP4	SNP4	Diploid		wt mt 	25.0%	35	5				
SNP5	SNP5	Diploid		mt	25.0%	35	5	~			
Group/Al	refix: SNP1	# of bea	ads in this grou	ар: 2				** Edit Bead Names			
Group N	ame: SNP1			Cha	nge Color	1					
Pi	oidy: 🖲 Diploid	C Ha	ploid C Oth	er Appl	y this Ploidy	to all groups	(loci)				
Allele Na	ime:			Cha	nge Color	Apply same of Apply	to all alleles in th order in each gro to all same name	e up. alleles			
Allele Ca	ll Parameters fo	r SNP1									
O Use	Relative Intensit	ty for Allele	call					100			
	Reportable Le	vel: 25.0	— % of total in	tensity				100			
	Intensity Thre	shold: 35	(MFI)					80			
C Inter	nsity based Allel	, le Call						60 33.333			
	Call anything I	bigger than	50 MF	'i as an Allele				20			
			Apply 1	to all groups (I	oci)			MFI Allele Ratio			

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.

 \Rightarrow The Open dialog box appears (Figure 6.13).

Open	? 🗙
Look jn: 隘	Settings 💽 🔶 🕂 📰 -
열 Grouop Se 열 Group Set 엘 Group Set	t 2.xml 1.xml 3.xml
File <u>n</u> ame:	<u>O</u> pen
Files of type:	MasterPlex Setting File (*.xml)

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
- Click the Save button . Alternatively, select File → Save Project from the menu bar.

 \Rightarrow The Save As dialog box appears (Figure 6.14).

Save As		?	×
Savejn: 障	SampleData		
HLA-A_B_ Sample1.g Sample2.g SampleBas SampleSmatrix	PR_BLD.gtp [p] tp e.gtp all.gtp		
File <u>n</u> ame:	Sample1_2.gtp	Save]
Save as type:	MasterPlex GT Project File (*.gtp)	✓ Cancel	

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 \rightarrow Save Project As from the menu bar.

 \Rightarrow 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 \cong . Alternatively, select File \rightarrow Open Project File from the menu bar.

 \Rightarrow The Open dialog box appears (Figure 6.15).



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

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 Master-Plex[™] GT application window (Figure 6.16).
 - \Rightarrow 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).

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

MasterPlex GT			
File Edit View Option Window Help			
MasterPlex	MasterF		
9T	9T		
Liteshi Osfara	-		
Hitachi Software	SampleData		
DIGITAL & GLOBAL	Ele Edit View Favorites Tools Help		
	🌀 Back 🔹 🕥 🕤 🏂 🔎 Search 📂 Folders		
	Address 🛅 C:\Program Files\HitachiSoft\MasterPlex GT 2.0\Se	mpleData	💙 🔁 GO
	Polders X Name - Reserved to the second se	Size Type Toble dtt IIE GTT File R_BLO-tt VEB Morrow 1 KB COTP File BAD-tt VEB Morrow 1 KB COTP File BAD-tt VEB Morrow 1 KB COTP File BAD-tt VEB Morrow 1 KB Core To File Morrow 2 KB Morrow 2 KB Morrow 2 KB Core To File Morrow 2 KB	ated Values File ated Values File rated Values File rated Values File rated Values File

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

CHAPTER RESULTS TABLES

The MasterPlex^m 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.)



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.
 - \Rightarrow 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.
 - \Rightarrow 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.

				Alleles at locus IA Alleles at locus IB					IB		
			Locus	IA.	1	1		в			
			Beads->	16124C	16126C	16129A	Anderson	16217C	16223T	16224C	Anderson
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
HI	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
<											>
				1				1			
				Blue	color	aradio	nte	Ped color gradiante			
				show	s rela	tive	1113	show	s rela	tive	1110

expression levels of expression levels of alleles at the first locus (IA)

alleles at the next locus (IB)

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:

 $RI_{a} = MFI_{a}/(MFI_{a} + MFI_{b}) \ge 100$ $RI_{b} = MFI_{b}/(MFI_{a} + MFI_{b}) \ge 100$

- To display the relative intensity view, click the $\frac{96}{10}$ button.
 - \Rightarrow 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	Andersor	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%	13.6%	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%	
 Image: A second s	40.0	0400	County Franks	35.5%	22.8%	7 1%	25 7%	17 9%	10 7%	A2 9% ¥	

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.

\Rightarrow 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.

%	• 🕅 🔳	<u>۽ ا</u>	≜↓ ⊨o 🛍	õ							
	1		Locus	IA				B			
			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
н	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.

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



7.2

The Statistics Table

In the Project Manager, the Statistics table displays MFI, backgroundadjusted 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).



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).

%	۱	📑 🖹 📶	2 ↓ 100	Í		
			Locus	r		
			Beads->	73A	73G	93A
Vell Name	Sample Name	Total Events	Notes			
D8	h16401 9	6000	Sample Empty	1	0	
F8	h16401 11	6000	Sample Empty	-1	0	
A8	h16401 6	6000	Sample Empty	2	0	
G8	h16401 12	4845	Sample Empty	-1	1	
D7	h16401 1	6000	Sample Empty	-1	2	
F7	h16401 3	6000		1	1	
G9	h16401 20	4000	County County	1	-1	
H7	h16401 5	Sort By Expressi	on	1	1	
B8	h16401 7	Sort By Sample		•	Ascending	
H8	h16401 13	Cluster Analysis	(Genotype/Expressio) (n	Descending	J
H9	h16401 21	Reset Sample So	rting	-1	5	
C7	h16401 blank	Open Bar Graph		-	-	
E7	h16401 2	Open Dar Graph		- 1	0	
C8	h16401 8	Open Scatter Pic	IC	0	1	
E8	h16401 10	Local Negative C	ontrol	0	0	
C9	h16401 16			0	1	
D9	h16401 17	Global Negative ·	Control	1	1	

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 2.
 - ⇒ The Typing table displays the reference sample in the first row and sorts the remaining samples (rows) by similar expression level in descending order.

CHAPTER 7 Results Tables

🏶 %	• 🖫 🔳	프 환 🛄	2↓ ∞ ∭	Ĩ					
			Locus	IA				в	
			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 Emptv	-	-	-	-	-	-
C4	beadsold	951	Sample Emptv	-	-	-	-	-	-
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	0010	Comple Emple	1 179	163	201	156	102	75
C2	47-2	Sort By Expression		150	140	169	143	111	71
C3	47-2d	Sort By Sample	,	216	192	204	192	135	90
G1	48-2	Cluster Analysis (Gen	otype/Expression)	137	114	134	107	78	61
G2	48-2	Reset Sample Sorting		138	128	152	120	83	60
G3	48-2d	Onen Bas Careb		124	105	133	112	96	72
E1	47-4	Open bar Graph		128	110	132	107	106	58
A2	48-4	Open Scatter Plot		148	170	212	148	167	96
E2	47-4	Local Negative Contr	ol	71	81	85	73	73	42
A3	48-4			- 177	149	164	151	149	92
E3	47-4d	Global Negative Cont	rol	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
НЗ	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 4 button
 - right-click the Typing table and click Allele Call in the shortcut menu that appears
 - select Function → Allele Call from the menu bar
 - \Rightarrow 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).

CHAPTER 7 Results Tables

		IA	IB	IC1	IC2	ID	IIA1	IIA2	IIB	IIC	IID		مامالا
[F1] 48-1	17 T	16126C	Anderson									E	Allele
[F2] 48-1	~	16126C	Anderson										names
[F3] 48-1d	~	16126C	Anderson										
[A1] beads only new													
[B4] beads new													
[C4] beadsold													
[62] 47-1	~	Anderson	Anderson										
[B3] 47-1d	~	Anderson	Anderson										
[B1] 47-1	7	Anderson	16217C Anderson										
[[1] 47-2 <	-		Anderson	Anderson	16311C 16311C 16320T	16362T					>	-	-Sample
[C2] 47-2	•		Anderson	Anderson	16311C 163 <u>11C 16</u> 320T	16362T							haploty
[C3] 47-2d	•		Anderson	Anderson (16311C 16311C 163207	16362T							
[G1] 48-2	~		Anderson	16294T	Anderson	16362T						1	
[G2] 48-2	~		Anderson	16294T	Anderson	16362T							
[G3] 48-2d	~		Anderson	16294T	Anderson	16362T							
[E1] 47-4	7									195C	263G		
[A2] 48-4	7		-							195C	263G		
[E2] 47-4	~									195C	263G		
[A3] 48-4	~									195C	263G		
[E3] 47-4d	~		-							195C	263G		
[A4] 48-4d	~									195C	263G	~	
											>		

Figure 7.10 Allele Call table

Genotype call displayed vertically.

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

🔳 Allele Call - Sample	92 (S	ample2.gt	p)							X	
BG 🎟 💵 🏘 🚺	AT	×									
Allele Call Allele Frequency	Ge	notype Frequ	ency Haplotype Fre	quency							
		IA	18	IC1	IC2	ID	IIA1	IIA2	118	^	
@ [F1] 48-1	V	16126C	Anderson								
C [F2] 48-1	~	16126C	Anderson								
[F3] 48-1d	~	16126C	Anderson								
[A1] beads only new											
[B4] beads new											
C [C4] beadsold											
C [B2] 47-1	~	Anderson	Anderson								Allele names
C [B3] 47-1d	~	Anderson	Anderson						-	-	/ tilolo hamoo
C [B1] 47-1	7	Anderson	16217C.Anderson			-	-	-			displayed
C [C1] 47-2	~		Anderson	Anderson	16311C,16311C 16320D	16362T					horizontally
C [C2] 47-2	~		Anderson	Anderson	16311C.16311C 16320T	16362T					nonzontany
C [C3] 47-2d	~		Anderson	Anderson	16311C.16311C 16320T	16362T					
C [G1] 48-2	7		Anderson	16294T	Anderson	16362T					
C [G2] 48-2	~		Anderson	16294T	Anderson	16362T					
C [G3] 48-2d	~		Anderson	16294T	Anderson	16362T					
C [E1] 47-4	~										
C [A2] 48-4	7										
C [E2] 47-4	7										
C [A3] 48-4	~										
C [E3] 47-4d	~										
C [A4] 48-4d	~										
C [D1] 47-3	7						73A	93G	Anderson		
C [D2] 47-3	~						734	936	Anderson	-	
< 11							1.961.9				
(1997) (1997)	_										4

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 (With-out Quotations)** from the shortcut menu that appears.

 \Rightarrow 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 \mathbf{X} 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).

CHAPTER 7 Results Tables

📕 Allele Call - Sample	e2 (S	ample2.gt	p)									×
BG 🎫 💶 🕸 🙀	AT .	- ×										
Allele Call Allele Frequency	/ Ge	notype Frequ	iency Haplo	type Frequer	icy							
		IA	IB	IC1	IC2	ID	IIA1	IIA2	IIB	IIC	IID	^
• [F1] 48-1		16126C	Anderson									
C [F2] 48-1	•	16126C	Anderson									
C [F3] 48-1d	~	16126C	Anderson									
[A1] beads only new												
[B4] beads new												
C [C4] beadsold												
C [B2] 47-1	•	Anderson	Anderson									
C [B3] 47-1d	~	Anderson	Anderson									
C [B1] 47-1	~	Anderson	16217C Anderson									
C [C1] 47-2	•		Anderson	Anderson	16311C 16311C 16320T	16362T						
C [C2] 47-2	•		Anderson	Anderson	16311C 16311C 16320T	16362T						
С [СЗ] 47-2d	•		Anderson	Anderson	16311C 16311C 16320T	16362T						
C [G1] 48-2	~		Anderson	16294T	Anderson	16362T						1
C [G2] 48-2	~		Anderson	16294T	Anderson	16362T						
C [G3] 48-2d	~		Anderson	16294T	Anderson	16362T						
C [E1] 47-4	~									195C	263G	
C [A2] 48-4	~									195C	263G	
C [E2] 47-4	~									195C	263G	
C [A3] 48-4	~									195C	263G	
C [E3] 47-4d	~									195C	263G	~
<											>	

💷 Allele Call - Sample	e2 (S	ample2.gt	р)										
BG III III 🐝 🚺	AT AC Ge	notype Frequ	 iency Haple	otype Freque	ncy								
		IA	IB	IC1	IC2	ID	IIA1	IIA2	IIB	IIC	IID		
[A1] beads only new													
• [1] 47M ✓ Anderson 16217C Anderson 16311C 16362T 73A 93G Anderson 195C 263G													
[2] 48M V 16126C Anderson 16294T Anderson 16362T 73G 93A 152C 195C 263G													
⊂ [3] 47M	~	Anderson	Anderson	Anderson	16311C 16311C 16320T	16362T	73A	93G	Anderson	195C	263G		
⊂ [4] 48M	~	16126C	Anderson	16294T	Anderson	16362T	73G	93A	152C	195C	263G		
⊂ [5] 47-d_M	~	Anderson	Anderson	Anderson	16311C 16311C 16320T	16362T	73A	93G	Anderson	195C	263G		
∩ [6] 48-d_M	•	16126C	Anderson	16294T	Anderson	16362T	73G	93A	152C	195C	263G		
C [B4] beads new													
C [C4] beadsold													

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 \mathfrak{P} . \Rightarrow 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.

 \Rightarrow The group number, well position, and sample names included in the merge are updated (Figure 7.13).

- 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.

 \Rightarrow The wells are merged in the Allele Call table (Figure 7.12).

 To toggle the Allele Call table between the pre- and post-merge views, click the Toggle Normal/Merged Well button ITT.

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 **11** button to display them.

🔳 Allele Call -	Sam	pleSm	all (Sa	mpleSn	nall.gtp)							
BG 🎫 📭 💐	*			×									
Allele Call Allele I	Freque	ency Ge	enotype F	requency	Haplot	ype Freq	uency						
		SNP1	SNP2	SNP3	SNP4	SNP5	SNP6	SNP7	SNP8	SNP9	SNP10		
@ [G1]6		wt,wt	wt,wt	wt,wt	wt,wt	wt,wt	wt,wt	wt,wt	wt,wt	wt,wt	wt,wt		
[H1]7 V wt,wt													
C [B2] 9	~	wt,wt	wt,wt	wt,wt	wt, mt	wt,wt	wt,wt	wt,wt	wt,wt	wt,wt	wt,wt		
C [A2] 8	~	wt,wt	wt,wt	wt,wt	wt,wt	wt,wt	wt,wt	wt,wt	wt,wt	wt,wt	wt,wt		
C [F1] 5	~	wt,wt	wt,wt	wt,wt	wt,wt	wt,wt	wt,wt	wt,wt	wt,wt	wt,wt	wt,wt		
C [B1] 1	•	wt,wt	wt,wt	wt,wt	wt,wt	wt,wt	wt,wt	wt,wt	wt,wt	wt,wt	wt,wt		
C [B3] 10	~	wt,wt	wt,wt	wt,wt	wt, mt	wt,wt	wt,wt	wt,wt	wt,wt	wt,wt	wt,wt		
C [E1] 4	•	wt,wt	wt,wt	wt,wt	wt,wt	mt, mt	mt, mt	wt,wt	wt,wt	wt,wt	wt,wt		
C [C1] 2	~	wt,wt	wt, <mark>mt</mark>	wt,wt	wt,wt	wt,wt	wt,wt	wt,wt	wt,wt	wt,wt	wt,wt		
C [D1] 3	~	wt,wt	wt, <mark>mt</mark>	mt, mt	wt, mt	mt, mt	mt, mt	wt,wt	wt,wt	wt,wt	wt,wt		
C [A1] no dna													
<											>		

Sample 6 selected for the reference

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 **AT** button (Figure 7.15).

📕 Allele Call -	San	pleSm	all (Sar	npleSm	all.gtp)							
BG 🎫 🎞 🍕	* 1			X									
Allele Call Allele	Freque	ency Ge	notype Fi	requency	Haplot	ype Frequ	iency						
		SNP1	SNP2	SNP3	SNP4	SNP5	SNP6	SNP7	SNP8	SNP9	SNP10		
[G1]6 ✓ wt.wt													
[H1] 7 ✓ wt.wt													
C [B2] 9	•	wt, wt	wt, wt	wt,wt	wt,mt	wt, wt	wt, wt	wt, wt	wt, wt	wt, wt	wt, wt		
C [A2] 8	•	wt, wt	wt, wt	wt,wt	wt, wt	wt, wt	wt, wt	wt, wt	wt, wt	wt, wt	wt, wt		
C [F1] 5	•	wt, wt	wt, wt	wt, wt	wt, wt	wt, wt	wt, wt	wt, wt	wt, wt	wt, wt	wt, wt		
C [B1] 1	I	wt, wt	wt, wt	wt,wt	wt, wt	wt, wt	wt,wt	wt, wt	wt, wt	wt, wt	wt,wt		
C [B3] 10	~	wt, wt	wt, wt	wt,wt	wt,mt	wt, wt	wt, wt	wt, wt	wt, wt	wt, wt	wt, wt		
C [E1] 4	I	wt, wt	wt, wt	wt,wt	wt, wt	mt,mt	mt,mt	wt, wt	wt, wt	wt, wt	wt,wt		
C [C1] 2	v	wt, wt	wt,mt	wt,wt	wt, wt	wt, wt	wt, wt	wt, wt	wt, wt	wt, wt	wt, wt		
C [D1] 3	•	wt,wt	wt, mt	mt,mt	wt,mt	mt,mt	mt,mt	wt, wt	wt, wt	wt, wt	wt,wt		
🔿 [A1] no dna	C [A1] no dna												

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.
 - \Rightarrow The Allele Call table displays '---' for samples that have fewer alleles called at a locus than the reference sample (Figure 7.16).

	I Allele Call - Sam	ole1	(Sam	ple1.gtp)								
	BG 🎫 🏧 🚯	AT AC		×								
	Allele Call Allele Frequer	cy (Senoty	pe Frequenc	y Haploty	pe Frequency						
Deference			П,	IA	IB	IC1	IC2	ID	IIB	пс	IID	
Reference —	• [D7] h16401 1				16223T	16292T/16295T	16311C	16362T				
sample	C [F7] h16401 3	•			16223T	16292T/16295T	16311C	16362T				
	C [G9] h16401 20	7			16223T	16292T/16295T	16311C					
	○ [G8] h16401 12				16223T	(-	16311C 16319A	16362T				
/	C [H9] h16401 21	~			16223T	1	16311C/16320T					
	○ [H8] h16401 13	•			16223T	<u> </u>	16311C 16311C/16320T	16362C				
/ —	C [D8] h16401 9	~			16223T	16294T	16311C	16362T				
/	C [E7] h16401 2	7			16223T		Anderson	16362T				
	C [C7] h16401 blank					\leq						
	C [C8] h16401 8	7			16223T	<u> </u>	Anderson	16362T				
Samples	C [A8] h16401 6	•			16223T	16304C	16311C	16362T				
with fewer	[B7] h16401 blank											
called alleles	[A4] h16236 blank											~
than the												11
reference												

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.

🔳 Allele Call - Sample	Allele Call - Sample2 (Sample2.gtp)											
BG 🎫 🎞 🌺 🚺	AT .	- ×										
Allele Call Allele Frequency	Ge	notype Frequ	ency Haplot	ype Fre	quency							
	Ė	IA	IB		IC1	IC2	ID	IIA1	IIA2	IIB		
C [F1] 48-1	~	16126C	Anderson			,						
C [F2] 48-1	~	16126C	Anderson			,					-	
C [F3] 48-1d	~	16126C	Anderson			,						
C [A1] beads only new												
[B4] beads new												
C [C4] beadsold												
C [B2] 47-1	•	Anderson	Anderson			,						
C [B3] 47-1d	•	Anderson	Anderson			,						
C [B1] 47-1	~	Anderson	16217C,And	lerson		<mark></mark> ,						
• [C1] 47-2			Anderson		Anderson	16311C,16311C 16320T	16362T					
[C2] Sort By Expre	ssion	le le			Anderson	16311C,16311C 16320T	16362T					
Cluster Analy	sis (Ge	enotype/Expre	ession)		Anderson	16311C,16311C 16320T	16362T					
C [G1] 48 Copy Table A	s Text				16294T	Anderson,	16362T				_	
C [G2] 48 Copy Table A	s Text	Without Qu	otations)		16294T	Anderson,	16362T				_	
Print Allele Ca	ill Tabl	e			16294T	Anderson,	16362T				- 1	
C [E1] 47 .						,					- 1	
C [A2] 48-4						,					- 1	
C [E2] 47-4	⊻					,	-				- 1	
[#3] 48-4	<u> </u>					,					- 1	
[E3] 47-40	M					,					- 1	
[H4]40-40						,			_		_	
C [D1] 47-3	M							73A	93G	Anderson	-	
[02]47-3	V					,		73A	93G	Anderson	_	
	_						_				2	
											1	

_ Reference sample (top row) for the sort

🔳 Allele Call - Sample	Allele Call - Sample2 (Sample2.gtp)											
BG 🎫 117 🍪 🚺	AL	<u>z x</u>										
Allele Call Allele Frequency	Ge	notype Frequ	ency Haplotype Fre	quency								
		IA	IB	IC1	IC2	ID	IIA1	IIA2	IIB			
• [C1] 47-2 /	2		Anderson	Anderson	16311C,16311C 16320T	16362T						
C [C2] 47-2	~		Anderson	Anderson	16311C,16311C 16320T	16362T						
C [C3] 47-2d	~		Anderson	Anderson	16311C,16311C 16320T	16362T						
C [G1] 48-2	~		Anderson	16294T	Anderson,	16362T				1		
C [G2] 48-2	~		Anderson	16294T	Anderson,	16362T						
C [G3] 48-2d	~		Anderson	16294T	Anderson,	16362T						
C [B2] 47-1	~	Anderson	Anderson		,							
C [B3] 47-1d	~	Anderson	Anderson		,					Ť.		
C [F3] 48-1d	~	16126C	Anderson		,					T		
C [B1] 47-1	1	Anderson	16217C, Anderson		,							
C [F1] 48-1	~	16126C	Anderson		,					1		
C [F2] 48-1	~	16126C	Anderson		,					Ť I		
C [A1] beads only new										-		
C [H1] 48-3	~				,		73G	93A	152C			
C [H3] 48-3d	~				,		73G	93A	152C	-		
C [H2] 48-3	~				,		73G	93A	152C			
C [D2] 47-3	7						73A	93G	Anderson	-		
C [D3] 47-3d	~						73A	93G	Anderson	1		
C [D1] 47-3	~				,		73A	93G	Anderson	+		
C [C4] beadsold	_									-		
C [E2] 47-4	7									-		
C [E1] 47-4	~									1		
C [A2] 48-4	~				····,···					~		
<	1.		-						2			
	_						_	_				

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 $\begin{bmatrix} A \\ B \\ B \end{bmatrix}$ 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).

🖪 Allele Call - Sample2 (Sample2.gtp)												
BG III III 🛞 🙀 👫 🗙												
Allele Call Allele Frequency	Ge	notype Frequ	ency Haplotype Fre	quency								
	IA IB IC1 IC2							IIA2	IIB	1		
@ [C1] 47-2	~	į.	Anderson	Anderson	16311C,16311C 16320T	16362T			į			
C [C2] 47-2	•		Anderson	Anderson	16311C,16311C 16320T	16362T						
C [C3] 47-2d	•		Anderson	Anderson	16311C,16311C 16320T	16362T						
C [G1] 48-2	~		Anderson	16294T	Anderson,	16362T						
C [G2] 48-2	~		Anderson	16294T	Anderson,	16362T						
C [G3] 48-2d	•		Anderson	16294T	Anderson,	16362T			Rofor	ance		
C [B2] 47-1	•	Anderson	Anderson		,				ricicit	51100		
C [B3] 47-1d	•	Anderson	Anderson		,							
C [F3] 48-1d	~	16126C	Anderson		,							
C [B1] 47-1	•	Anderson	16217C, Anderson		,							
C [F1] 48-1	~	16126C	Anderson		,							
C [F2] 48-1	~	16126C	Anderson		,							
[A1] beads only new												
C [H1] 48-3	~				,		73G	93A	152C	:		
C [H3] 48-3d	~				,		73G	93A	152C	:		
C [H2] 48-3	~				,		73G	93A	152C			
C [D2] 47-3	~				,		73A	93G	Anderson			
C [D3] 47-3d	~				,		73A	93G	Anderson			
C [D1] 47-3	~				,		73A	93G	Anderson			
C [C4] beadsold												
C [E2] 47-4	~				,							
C [E1] 47-4	~				,							
C [A2] 48-4	~				,					~		
<	-		-		•					2		

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.

 \Rightarrow The table information is copied to the system clipboard.

💻 Allele Call - Sample2 (Sample2.gtp) 📃 🗖 🔀											
BG 💵	ITT 🎲 🚺 👫	×									
Allele Ca	Allele Frequency Genoty	pe Frequency	Haplotype	Frequency							
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%	<u>×</u>							
				11							

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.

💶 Allele Call - Sample2 (Sample2.gtp) 📃 🔲 🔀												
BG 💵	BG 🏢 🎹 🐝 🗰 👯 🗙											
Allele Ca	Allele Frequency Genoty	pe Frequency	Haplotype	Frequency								
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	736	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.

\Rightarrow The table information is copied to the system clipboard	rd.
-----------------------------------------------------------------------	-----

1	💶 Allele Call - Sample2 (Sample2.gtp)												
BG III III 🛞 III 🔝 🗙													
6	Allele Call Allele Frequency Genotype Frequency Haplotype Frequency												
[IA 🗸	IB	IC1	IC2	ID	IIA1	IIA2	IIB	IIC	IID	Frequency	%	
ľ		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%	
f													

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 sutton.
 - \Rightarrow 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).

🔳 Homo	logy Plot - Sar	nple2												×
Homology	Homology Table Homology Chart													
		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	0.98959	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	
61	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. \Rightarrow The window displays the Homology chart (Figure 7.23).

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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.

 \Rightarrow 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.



 \Rightarrow 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.

CHAPTER 7 Results Tables

CHAPTER GRAPHS

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.

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.

Table 8.1 MasterPlex[™] GT graphs

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 8.3Heat mapAlleles from left to right.

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

Application Options	
General Background Clustering Tool Plugins	
Read Name Style	
C Locus Name + Allele Name	
C Original Bead Name	
Start Up Window After Data Loading	
Show Table View	
C Show Graph View	
Table View Gradation Background	
Use Allele Call Color for gradation background	
Group Color #1 Change Color	
Group Color #2 Change Color	
Leatman Ontions	
Heatmap Bar Size: 3 💽 Pixel(s) / Allele	
OK Reset All Cancel	

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 $\ensuremath{\mathbb{RI}}$ 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.

- \Rightarrow 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.
 - \Rightarrow 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.

 \Rightarrow 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

Default	То	Click
3-dimensional (3D) graph	Toggle the view between 3D and 2D view.	7
Show name tags for called alleles	Hide or unhide the name tags.	<mark>Б</mark> а
Display the graph legend	Hide or unhide the legend.	<u>‡</u> ⊒
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	LØ
Display MFI data	Display relative intensity data (RI).	RI
	Return the display to MFI data.	MEI
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.	ABC
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.	ABC
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.	<u>סם</u> ֿם

Table 8.2 Graph display options

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.

y-axis slider



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 Hx1.0 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 $\vee \times 1.0$ 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).

 \Rightarrow 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).

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.





Figure 8.11 Multi Compare graph

Rectangle specifies the area to be magnified.

 $[\]Rightarrow$ The Project Window displays the selected graph area (Figure 8.12).



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.

 \Rightarrow The mouse pointer changes to a $\stackrel{\bullet}{=}$.

- 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 yaxis 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.
 - \Rightarrow 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.
 - \Rightarrow 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.

 \Rightarrow A pop-up tool tip displays the allele name.

6. To display all of the allele name tags, click the graph.

 \Rightarrow 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.



Sample Name list

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 userselected 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.
 - \Rightarrow 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.

9 CLUSTER ANALYSIS

This chapter explains how to apply a cluster analysis to the samples and display a sample dendrogram. The MasterPlex^m 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)

CHAPTER 9 Cluster Analysis



Figure 9.1 Project Manager

- 3. Click the Show Dendrogram toolbar button D.
 - \Rightarrow 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 ³ is not available in the Allele by Allele tab.

Sample	Small Cl	ustering	g Tool	×						
N.N.Min	F.N.Max	Median	Centroid							
B.G.Link	Ward's	Flexible	-0.25	•						
Genotype C Expression										

Figure 9.2 Clustering Tool window



Figure 9.3 Dendrogram in the MultiGraph view

			1	Locus	SNP1		SNP2		SNP3		SNP4	
				Beads->	wt	mt	vvt	mt	wt	mt	vvt	rnt
	vVell Name	Sample Name	Total Events	Notes								
1	B1	1	2582		50	0	50	8	45	21	36	
	F1	5	2860		354	21	475	39	656	48	576	
	A2	8	2802		614	42	593	81	1080	48	822	
	G1	6	2711		307	29	548	40	913	95	756	
י ה	H1	7	2517		409	35	702	106	1244	194	704	
L	C1	2	2652		605	44	725	645	368	68	442	
	B2	9	2736		450	33	567	37	700	80	370	
1	B3	10	3100		334	31	579	47	967	114	783	
	D1	3	2924		346	34	616	661	20	981	527	· · · ·
	E1	4	3103		486	35	607	41	932	80	583	
	A1	no dna	21		-	-	-		-	-	-	
	Sample Small Clustering Tool X NNMin FNMax Medan 0.23 Cer B.o.Link Wards Flexible © Genotype © Expression											

Figure 9.4 Dendrogram in the Typing table

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

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

CHAPTER **GENOTYPING USING A LOOKUP** 10 TABLE

The MasterPlex^m *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.

%	💌 🕎 📗	3	<u>II</u> ≙↓	»	©						
			Locus	A				1	1		в
			Beads->	01	02	03	04	05	Std1	Std2	01
v/vell Name	Sample Name	Total Events	Notes								
A1	blank	5200		-	-	-	-	-	-	-	-
B1	id0001	5866		272	272	73	8	74	2469	1486	268
C1	id0002	4680		72	272	173	58	274	3217	1100	268
D1	id0003	4973		272	22	273	58	274	614	2200	268
E1	id0004	4970		272	272	273	258	24	1909	2449	18
F1	id0005	5015		272	272	273	258	274	1099	3934	18
G1	id0006	5152		272	272	73	58	24	1459	2456	268
HI	id0007	5660		272	272	273	258	24	1506	4798	268
A2	id0008	4845		272	272	73	258	274	1060	1070	268
B2	id0009	5591		22	72	273	258	274	2059	1929	68
C2	id0010	5016		272	72	73	258	274	2359	2487	18
D2	id0011	4881		272	272	273	58	24	2459	3911	268
E2	id0012	5216		72	272	273	58	274	2414	2246	268
F2	id0013	5639		272	272	273	258	74	2410	1931	268
G2	id0014	5035		272	272	73	258	274	1509	957	268
H2	id0015	6533		272	72	273	258	274	2199	2286	268

Ele Edit Yew Fund

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😑 🖢 🔟 🖉 🕬 🎬 🎯

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.

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.

Oroup s	Save sets	ig 65	Inport Setting	•	Cancel	
F Per I⊽ Use	arteter setup fi group color to	r the indu	idual bead. M d Alete Call Table	inimum Events: 20	count for each be	ed Lookup Te
Pretix	Group Name	Type	Lookup Table	Aleie Name	%Report Intens	ityCall
8	в	Other		01 02 03 04 2841 2842 01 02 02	25.0% 35	
<						
Group Group	Prefix A	10	I beads in this group	x 7 Change Colo		* Edit Dea
Allele 1	Noldy: C DeA	NU (Heplad I Offe	Change Colo	Apply to all	ideles in the
Allele C C Us	all Parameters e Relative Inter Reportable Interatly Th ensity based A Call anythin	tor A sity for Al evet estroits tele Call g bigger ti	lele call 50 % of total into 6970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 9970 99700 9970 9970 9970 9970 9970 9970	ac an Alele	\mathbf{i}	

Clieber to be to					Locus			A						
Click a tab to				-	Beads->	-	-	01	02	03	04	05	Std1	Std2
coloct a la cuia am	Well Name	Sample Name	Total Events	Notes	Type(A)	Åm	N O							
select a locus or	A1	blank	5200	-	No Matches			110	110	20		50	1000	101
the bloodture	81	100001	4690	-	A UUS. A UTU	0	13	25	85	54	53	249	1000	101
the bloodtype.	DI	W0002	4000	-	A 007		15	443	36	445	26	125	1000	101
	E1	100003	4970	-	A 009 A 010	0	12	143	142	143	105	10	1000	101
	F1	id0005	5015		A 004, A 009	5	21	241	247	248	66	70	1000	101
	G1	id0006	5152	-	A 005 A 010	0	17	186	186	50	24	10	1000	101
	HI	id0007	5660		A 009, A 010	0	12	181	181	181	54	5	1000	101
	A2	id0008	4845		A 004, A 009	5	21	257	257	69	241	256	1000	101
	B2	id0009	5591		A 007. A 009	0	4	11	35	133	134	142	1000	101
	C2	id0010	5016		A 006	1	8	115	31	31	104	110	1000	10
	D2	id0011	4881		A 005. A 010	0	17	111	111	111	15	8	1000	10
	E2	id0012	5216		A 005. A 007	0	15	30	113	113	26	122	1000	101
	F2	id0013	5639		A 009. A 010	0	12	113	118	113	134	38	1000	101
	G2	id0014	5035		A 004, A 006	4	15	180	180	48	270	286	1000	101
	H2	id0015	6533		A 006. A 009	2	- 11	124	33	124	113	120	1000	101
	Click:	Ĩ	to view	by lo	ocus.									
		Ô	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 🆃.

 \Rightarrow The Parameter Setting dialog box appears (Figure 10.4).

Parameter Setting							
Group set: 📕	ave setting as	Import Setting	•	Cancel	ок		
Parameter setup for the individual bead. Minimum Events: 20 count for each bead Use group color for Chart and Allele Call Table Lookup Table							
Prefix Grou	oName Type	Lookup Table	Allele Name	%Report Inter	nsity Call Inten. 🔼		
A A B B	Other Other	HLA A v1.10	01 02 03 04 05 Std1 Std2 01 02 02		50		
Group/Allele Identifier # of beads in this group: 7 # Edit Bead Names							
Group Name:	A		Change Cold	r			
Ploidy: C Diploid C Haploid C Other Apply this Ploidy to all groups (loci)							
Allele Name:			Change Cold	r Apply to al same orde	l alleles in the r in each group. I same name alleles		
Allele Call Parameters for A Use Relative Intensity for Allele call Reportable Levet 25.0 % of total intensity Intensity Threshold: 35 (MFI) Intensity based Allele Call Call anything bigger than 50 MFI as an Allele Apply to all groups (loci)							

Figure 10.4 Parameter Setting dialog box

c. Click Lookup Table.

 \Rightarrow 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.

💶 Lookup Table Selection						
Entry Name	Last Modified	А	B DR BL		BLD	Edit Selected Entry
(PROJECT)		HLA A Ver1.10	HLA B Ver1.10	HLA DR Ver1.10	Blood Type Ver1.00	Create New Lookup Entry
HLA Lookup Table	10/31/2003 12:56:50 PM	HLA A Ver1.10	HLA B Ver1.10	HLA DR Ver1.10	Blood Type Ver1.00	Delete Selected Entry
						Select Latest Tables
						Unselect All
						ок
						Cancel

Figure 10.5 Lookup Table Selection window

This window shows the lookup tables installed in the MasterPlex ${}^{\rm \tiny M}$ 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.
ОК	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.
- Click the Parameter Setting button [™].
 ⇒ The Parameter Setting dialog box appears (Figure 10.7).
| 🔳 Parame | ter Settir | ıg | | | | | | | | |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------|-------------|------------|---------------------------|--------------------------------------------|--------------------------------------------------------------------|---------------------|--|--|--|
| Group set: | | ſ., | | • | [| Cancel | ок | | | |
| S | Save setting as | | | | | | | | | |
| □ Parameter setup for the individual bead. Minimum Events: 20 count for each bead ✓ Use group color for Chart and Allele Call Table Lookup Table | | | | | | | | | | |
| Prefix Grou | ip Name | Туре | Lookup ' | Table | Allele Name | %Report Inte | nsity Call Inten. 🔨 | | | |
| A A | | Other | HLA A V | 1.10 | | | 50 | | | |
| | | | | | 01
02
03
04
05
Std1
Std2 | | | | | |
| в в | | Other | HLA B v | 1.10 | | | 50 | | | |
| | | | | | 01 | | | | | |
| < | | | | | 02 | | | | | |
| -Group (Allele | Identifier | | | | | | | | | |
| Group Prefix | A | # of b | eads in th | nis group: i | , | * | * Edit Bead Names | | | |
| Group Name | A | | _ | | Change Cold | r | | | | |
| Ploidy | : C Diploid | 0 | Haploid | Other | Apply this Plo | idy to all groups (lo | ci) | | | |
| Allele Name: | | | _ | | Change Cold | Change Color Apply to all alleles in the same order in each group. | | | | |
| Allele Call Pa | rameters for | r A | | | | | | | | |
| C Use Rela | ative Intensit | y for Allel | e call | | | | | | | |
| Re | portable Le | vel: 25 | .0 % of | total inten: | sity | | | | | |
| Int | ensity Thre | shold: 35 | (| (MFI) | | | | | | |
| Intensity | based Allel | e Call | | | | | | | | |
| Ca | all anything b | bigger that | n 50 | MFLas | an Allele | | | | | |
| | | | | Apply to a | ll groups (loci) | | | | | |
| | | | | | | | | | | |

Figure 10.7 Parameter Setting dialog box

3. Click Lookup Table.

 \Rightarrow The Lookup Table Selection window appears (Figure 10.8).

🔳 Lookup Tal						
Entry Name	Last Modified	А	В	DR	BLD	Edit Selected Entry
(PROJECT)		HLA A Ver1.10	HLA B Ver1.10	HLA DR Ver1.10	Blood Type Ver1.00	Create New Lookup Entry
HLA Lookup Table	10/31/2003 12:56:50 PM	HLA A Ver1.10	HLA B Ver1.10	HLA DR Ver1.10	Blood Type Ver1.00	Delete Selected Entry
						Select Latest Tables
						Unselect All
						ок
						Cancel

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.

 \Rightarrow The New Lookup Table Entry dialog box appears (Figure 10.9).

New Lookup Table Entry								
Lookup table name:								
HLA Lookup Table								
OK Cancel								

Figure 10.9 New Lookup Table Entry dialog box

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

💷 Lookup Table Editor									-	
					Expo	ort	Apply	·	Reset All	Ce
A B B C DR BLD										
Locus Setting Name HLA A	Vers	ion 1.1	\$	Group	Name	B,			Reset	
Task 🔐 🔐	1									
Type Nam	e Short Name	Color	Frequency	A_01	A_02	A_03	A_04	A_05	A_Std1	A_Std2
Туре										
Standard Beads										
Cross Talk										
General										
										>
										1

Click a task button to change the view in the Lookup Table Editor.

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).

1. Click a tab to select the locus. 2. Enter a name for the selected locus or blood type.

١

3. Enter a version number for the selected locus.



The locus setting name and version number entered in the Lookup Table Editor (top) will appear in the Lookup Table Selection window (bottom).

🔳 Lookup Tal						
Entry Name	Last Modified	А	В	DR	BLD	Edit Selected Entry
(PROJECT)		HLA A	HLA B	HLA DR	Blood Type	
LILATERIA Table		Ver1.10	Ver1.1U	Ver1.10	Ver1.00	Create New Lookup Entry
ных соокир таріе	10/31/2003 12:56:50 PM	Ver1.10	Ver1.10	Ver1.10	Ver1.00	Delete Selected Entry
		\sim				· · · ·
						Select Latest Tables
						ок
						Cancel

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 and select Add New from the shortcut menu that appears.

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

New Type Name Entry								
Type Name:								
A 001								
OK Cancel								

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.

 \Rightarrow The Edit Type Name Entry dialog box appears (Figure 10.14).

Edit Type Name Entry								
Edit Type Name:								
A 002								
	1							
OK Cancel								

Figure 10.14 Edit Type Name Entry dialog box

- To delete a type name, select the type name and click the Delete button T. 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.
 - \Rightarrow The color palette appears (Figure 10.15).

Color	? 🗙
Basic colors:	
Custom colors:	
Define Custom Colors >>	,
OK Cancel	

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.
 - \Rightarrow 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.
 - \Rightarrow 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.

							[Export	Apply	Res	et All Ci
	BLD										
Locus Setting Name	ILA A	Versio	n 1.1	\$	Group I	Name A			Reset		
Task	節門音										
	Standard	Base MFI	Cutoff	Enable	A_01	A_02	A_03	A_04	A_05	A_Std1	A_Std2
Туре	A_Std1	1000	300	TRUE	1	1	1				
<u> </u>	A_Std2	1000	300	TRUE				 Image: A second s	 Image: A second s		
Standard Beads											

Click the Standard Beads button to change the view in the Lookup Table.

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
 - \Rightarrow The Standard Beads view for the selected locus or blood type appears (Figure 10.18).

📕 Lookup Table Edit	tor										
								Export	Apply	Res	et All C
	BLD										
Locus Setting Name		Versi	ion 0.0	\$	Group I	Name 🔺			Reset		
Task											
	Standard	Base MFI	Cutoff	Enable	A_01	A_02	A_03	A_04	A_05	A_Std1	A_Std2
Туре											
Standard Beads											
Cross Talk											
General											
CONDIG											

Figure 10.18 Lookup Table Editor, Standard Beads view

3. Click the Add New button 2. Alternatively, right-click the Lookup Table Editor and select Add New from the shortcut menu that appears.

 \Rightarrow The New Standard Bead Name Entry dialog box appears (Figure 10.19).

New StandardBead Name Entry	
StandardBead Name	
ок	Cancel

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.

 \Rightarrow The standard is added to the Lookup Table Editor (Figure 10.20).

💶 Lookup Table Ed	itor										
							I	Export	Apply	Res	et All
A B DR	BLD										
Locus Setting Name		Versio	n 0.0	\$	Group	Name 🔺			Reset		
Task											
	Standard	Base MFI	Cutoff	Enable	A_01	A_02	A_03	A_04	A_05	A_Std1	A_Std2
Туре	A_Std1	1000	300	FALSE							
Standard Beads											
Cross Talk											
General											

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.

Export Apply Reset Al A B D.R BLD Locus Setting Name Version 0.0 Croup Name Reset Tesk Standard Base MFI Catolf Enable A_D1 A_D2 A_D3 A_D4 A_D5 A_Std1 A_S0 Type Track Trac	💷 Lookup Table E	ditor										
									Export	Apply	Res	et All
Locus Setting Name Version 0.0 Croup Name A Reset Task	A B D	R 🛛 🗖 BLD 🗍										
Tesk Standard Base MFI Cutoff Enable A_01 A_02 A_03 A_04 A_05 A_5td1 A_5td1	Locus Setting Name		Versio	n 0.0	\$	Group I	Name 🔺			Reset		
Standard Base MFI Cutoff Enable A_01 A_02 A_03 A_04 A_05 A_5k1	Task											
Type A_Std1 1000 300 FAISE V FALSE Standard Beads Cross Talk General		Standard	Base MFI	Cutoff	Enable	A_01	A_02	A_03	A_04	A_05	A_Std1	A_Std2
TRUE Standard Boods Cross Talk Ceneral	Туре	A_Std1	1000	300	FALSE	~						
ITALSE					TRUE							
Cross Talk	Stendard Beede				FALSE							
Cross Talk General	Stanuaru Beaus											
Cross Talk General												
General	Cross Talk											
General												
	General											

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).

📕 Lookup Table Ed	litor								
						Export	Apply	Reset	All C
	(🗖 BLD								
Locus Setting Name		Vers	sion 0.0	\$	Grou	oName 🗛			Reset
Task									
	Standard	Enable	A_01	A_02	A_03	A_04	A_05	A_Std1	A_Std2
Туре	A_Std1	TRUE	1	-	 ✓ 				\checkmark
Standard Beads									
Cross Talk									
General									
	<								

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.
- 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 **T**. 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:

Corrected MFI_A = Original MFI_A - (0.1* MFI_B) - (0.05* MFI_C)

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

 \Rightarrow The Cross-talk view for the selected locus or blood type appears (Figure 10.23).

🔳 Lookup Table Ec	litor									
						Export		Apply	Reset All	Ca
	BLD									
Locus Setting Name	HLA A	Version	1.1	\$	Gro	up Name	Α,		Rese	et 🔤
Task										
	A_01	A_02	A_03	A_04	A	_05	A_Std1	A_Std2		
Туре										
Standard Beads										
Cross Talk										
General										

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 2. Alternatively, right-click the Lookup Table Editor and select Add New from the shortcut menu that appears.
 - \Rightarrow The New Standard Bead Name Entry dialog box appears (Figure 10.19).

New TargetBead Entry	
TargetBead Name	
A 00	.
<u> 0_02</u>	<u> </u>
ок	Cancel

Figure 10.24 New Target Bead Entry dialog box

- Click the drop-down arrow and make a selection from the dropdown 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).

🔳 Lookup Table Edi	itor									
						Export	/	Apply	Reset All	Ca
A B DR	BLD									
Locus Setting Name			Version 0).0	G	roup Name	A		Re	set
Task	F I	F								
		A_01	A_02	A_03	A_04	A_05	A_Std1	A_Std2		
Туре	A_02	0	0	0	0	0		0	0	
Standard Beads										
Cross Talk										
General										
										1

Figure 10.25 Lookup Table Editor, Cross-talk view

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

🔳 Lookup Table Ed	litor											
							Export	A	pply	Res	et All	С
	BLD											
Locus Setting Name	HLA A		Versio	n 1.1	\$	Gro	oup Name	A			Reset	t
Task		F										
		A_01	A_02	A_03	A_04	F	A_05	A_Std1	A_Std2			
Туре	A_01		0	.03	0	0	.02)	0		
Chanada wal Basada												
Standard Beads												
Cross Talk												
General												

Figure 10.26 Lookup Table Editor, Cross-talk view

In Figure 10.26, the software computes $\rm MFI_{A\,01}$ 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 **T**. Alternatively, rightclick 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

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

🔳 Lookup Table E	ditor					
			Export	Apply	Reset All	C
A B DF						
Locus Setting Name	HLA A	Version 1.1	\$ Group Name		Res	et
Task						
	Parameter	Value				
Туре	Ploidy	Diploid				
	Unmatch Caption	No Matches				
	Comma Split	TRUE				
Standard Beads	Include Standard	TRUE				
Cross Talk						
General						

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 geno- type. 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 Typ- ing 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 $^{\textcircled{}}$. \Rightarrow The Parameter Setting dialog box appears (Figure 10.28).

🔳 Parame	ter Setting								
Group set:	ave setting as	Import	▼ Setting		Cancel	ок			
Parameter setup for the individual bead. Minimum Events: 20 count for each bead Use group color for Chart and Allele Call Table Lookup Table									
Prefix Grou	oName Ty	pe Look	cup Table	Allele Name	%Report Int	ensity Call Inten. 🔼			
A A	Oti Oti	her HLA her HLA	.A v1.10 .B v1.10	01 02 03 04 05 Std1 Std2 01 01 02 r2		50			
<u> </u>						2			
-Group/Allele I Group Prefix:	A A	# of beads	in this group: 7	,		** Edit Bead Names			
Group Name:	A			Change Colo	r				
Ploidy:	C Diploid	C Haplo	id 💽 Other	Apply this Plo	idy to all groups (li	oci)			
Allele Name:				Change Colo	Apply to same orc	all alleles in the ler in each group. all same name alleles			
-Allele Call Par	ameters for A tive Intensity fr portable Level ensity Thresho based Allele C II anything bigg	or Allele call : 25.0 9 old: 35 call ger than 50	6 of total intens (MFI) MFI as Apply to a	sity : an Allele Il groups (loci)					
						//			

Figure 10.28 Parameter Setting dialog box

3. Click Lookup Table.

 \Rightarrow The Lookup Table Selection window appears (Figure 10.29).

🔳 Lookup Tal						
Entry Name	Last Modified	A	В	DR	BLD	Edit Selected Entry
(PROJECT)		HLA A Ver1.10	HLA B Ver1.10	HLA DR Ver1.10	Blood Type Ver1.00	Create New Lookup Entry
HLA Lookup Table	10/31/2003 12:56:50 PM	HLA A Ver1.10	HLA B Ver1.10	HLA DR Ver1.10	Blood Type Ver1.00	Delete Selected Entry
						Select Latest Tables
						Unselect All
						ок
						Cancel
					>	

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).

Lookup Table E	ditor										.)[0
						Ex	port	Арр	ly	Reset A	
A B B	R 🗌 🗖 BLD 🛛										
Locus Setting Name	HLA A	Vers	ion 1.1	\$	Group	pName	A			Reset	
ask											
	Type Name	Short Name	Color	Frequency	A_01	A_02	A_03	A_04	A_05	A_Std1	A_St
Туре	A 001	001		0.001		+		+		+	+
	A 002	002		0.0001	+	+	+		+	+	+
	A 003	003		0.01		+				+	+
Standard Beads	A 004	004		0.5	+	+			+	+	+
	A 005	005		0.006		+	+			+	+
Crease Tells	A 006	006		0.51	+			+	+	+	+
Cruss Taik	A 007	007		0.022					+	+	+
	A 008	008		0.01	+		+		+	+	+
General	A 009	009		0.4			+	+		+	+
	A 010	010		0.0006	+	+				+	+

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
Туре	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 P. \Rightarrow The Parameter Setting dialog box appears (Figure 10.31).

📕 Paramete	r Setting						
Group set: Sav	e setting as	Import Setting	•	Cancel	ок		
Parameter setup for the individual bead. Minimum Events: 20 count for each bead Use group color for Chart and Allele Call Table Lookup Table							
Prefix Group N	lame Type	Lookup Table	Allele Name	%Report Intensity	/ Call Inten. 🔼		
A A	Other	HLA A v1.10			50		
			01 02 03 04 05 Std1 Std2				
B B	Other	HLA B v1.10			50		
			01 02				
<			02				
-Group (Allele Ide	ntifier						
Group Prefix:	# 01	f beads in this group:	7	** Edi	t Bead Names		
Group Nama:	7	_	Chapge Color	1			
Ploidy: (Diploid C	Haploid 📀 Other	Apply this Ploid	y to all groups (loci)			
			Classes Calar	Apply to all alle	eles in the		
Allele Name.			Criange Coor	same order in Apply to all sa	each group. me name alleles		
Allele Call Param	eters for A		Change color	same order in Apply to all sa	each group. me name alleles		
Allele Call Paran	eters for A	lele call		Apply to all sat	each group. me name alleles		
Allele Call Paran C Use Relativ Repo	eters for A e Intensity for Al rtable Level:	lete call	nsty	same order in Apply to all sat	each group. me name alleles		
Allele Call Paran Allele Call Paran C Use Relativ Repo Inten:	eters for A e Intensity for Al rtable Levet:	lele call 15.0 % of total inter 15.0 (MFI)	Criange Coor	Apply to all sar	each group. me name alleles		
Allele Call Param C Use Relativ Repo Intensity ba	eters for A e Intensity for Al rtable Level: sity Threshold: sed Allele Call	lele call 5.0 % of total inter 35 (MFI)	Criainge Color	Apply to all sa	each group, me name alleles		
Allele Call Param C Use Relativ Repo Intens C Intensity ba Call a	e Intensity for A rtable Levet:	lele call 15.0 % of total inter 15	is an Allele	same order in Apply to all sa	each group, me name alleles		
Allele Call Paran C Use Relativ Repo Inten- C Intensity ba Call a	eters for A e Intensity for Al rtable Levet: sity Threshold; sed Allele Call nything bigger th	lele call 15.0 % of total inter 15.0 (MFI) 14.0 50 MFI a Apply to :	is an Allele	same order in a	each group. me name alleles		

Figure 10.31 Parameter Setting dialog box

3. Click Lookup Table.

 \Rightarrow The Lookup Table Selection window appears (Figure 10.32).

🔳 Lookup Tal	ole Selection					
Entry Name	Last Modified	А	В	DR	BLD	Edit Selected Entry
(PROJECT)		HLA A Ver1.10	HLA B Ver1.10	HLA DR Ver1.10	Blood Type Ver1.00	Create New Lookup Entry
HLA Lookup Table	10/31/2003 12:56:50 PM	HLA A	HLA B	HLA DR	Blood Type	
		Ver1.10	Ver1.10	Ver1.10	Ver1.00	Delete Selected Entry
						Select Latest Tables
						Unselect All
						ОК
						Cancel

Figure 10.32 Lookup Table Selection window

4. Click the table that you want to export, and click Edit Selected Entry.

Lookup Table E	ditor										
						Ex	port	Арр	oly	Reset A	.u
A B B											
Locus Setting Name	HLA A	Vers	ion 1.1	\$	Group	Name	A			Reset	
ask											
	Type Name	Short Name	Color	Frequency	A_01	A_02	A_03	A_04	A_05	A_Std1	A_Std2
Type	A 001	001		0.001		+		+		+	+
	A 002	002		0.0001	+	+	+		+	+	+
	A 003	003		0.01		+			0	+	+
Standard Beads	A 004	004		0.5	+	+			+	+	+
	A 005	005		0.006		+	+			+	+
Cross Talk	A 006	006		0.51	+			+	+	+	+
Cross Taik	A 007	007		0.022					+	+	+
	A 008	008		0.01	+		+		+	+	+
General	A 009	009		0.4			+	+		+	+
	A 010	010		0.0006	+	+				+	+

 \Rightarrow The Lookup Table Editor appears (Figure 10.33).

Figure 10.33 Lookup Table Editor

5. Click Export.

 \Rightarrow The Export Wizard appears (Figure 10.34).

ncheck All

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.
 - \Rightarrow The Save As dialog box appears (Figure 10.35).

Save As			? 🛛
Savejn: 🗀	SampleData	- + E i	* 💷 •
File <u>n</u> ame:	HLA Lookup Table.gtt		<u>S</u> ave
Save as type:	Lookup Table (*.gtt)	•	Cancel

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 tabdelimited text file.

- 1. Right-click the lookup table and select **Copy as Text** from the shortcut menu that appears.
 - \Rightarrow 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).

🔳 Lookup Tal						
Entry Name	Last Modified	A	В	DR	BLD	Edit Selected Entry
(PROJECT)		HLA A Ver1.10	HLA B Ver1.10	HLA DR Ver1.10	Blood Type Ver1.00	Create New Lookup Entry
HLA Lookup Table	10/31/2003 12:56:50 PM	HLA A Ver1.10	HLA B Ver1.10	HLA DR Ver1.10	Blood Type Ver1.00	Delete Selected Entry
						Select Latest Tables
						Unselect All
						ок
						Cancel
					>	

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
- To open a Luminex results file (.csv), click the Open CSV File button . Alternatively, select File → Open CSV File from the menu bar.

 \Rightarrow The Open dialog box appears (Figure 10.37).

Select the .csv of interest and click Open.
 ⇒ The Typing table displays the results data.

Open					? 🔀
Look jn: 🔎	SampleData	•	← 🖻) 📥	
HLA-A_B_C Sample1.c: Sample2.c: SampleBas	DR_BLD.csv sv sv e.csv all.csv				
File <u>n</u> ame:	HLA-A_B_DR_BLD.csv				<u>O</u> pen
Files of type:	Luminex Data file (*.csv, *.mdb)		•		Cancel

Figure 10.37 Open dialog box

🔳 MasterPlex GT - [Typing - HLA	MasterPlex GT - [Typing - HLA.A_B_DR_BLD ()									
Ele Edit View Function Option	Window Help									_ 8 ×
Multi Croph				Locus	A					
Inditi Graph				Beads->	01	02	03	04	05	Std1
	v/vell Name	Sample Name	Total Events	Notes						
	A1	blank	5200		-	-	-	-	-	-
	B1	id0001	5866		272	272	73	8	74	2469
	<u>C1</u>	id0002	4680		72	272	173	58	274	3217
	D1	id0003	4973		272	22	273	58	274	614
	E1	id0004	4970		272	272	273	258	24	1909
	F1	id0005	5015		272	272	273	258	274	1099
	G1	id0006	5152		272	272	73	58	24	1459
	H1	id0007	5660		272	272	273	258	24	1506
MFI Adjusted MFI Count	A2	id0008	4845		272	272	73	258	274	1060
Bead Ave SD CV%	82	id0009	5591		22	72	273	258	274	2059
	C2	id0010	5016		272	72	73	258	274	2359
	D2	id0011	4881		272	272	273	58	24	2459
× 02 0 0.0 0.0%	E2	id0012	5216		72	272	273	58	274	2414
A 05 0 0.0 0.0%	F2	id0013	5639		272	272	273	258	74	2410
A U4 0 0.0 0.0%	G2	id0014	5035		272	272	73	258	274	1509
A 05 0 0.0 0.0%	H2	id0015	6533		272	72	273	258	274	2199 👻
🛛 A Std1 📔 0 0.0 0.0% 💆	•									•

Figure 10.38 MasterPlex GT application window

3. Click the **Parameter Setting** button 🏶.

 \Rightarrow The Parameter Setting dialog box appears (Figure 10.39).

📕 Parameter Set	ting			×		
Group set:		•		٦		
Save setti	ng as Import Setting		Cancel UK			
Parameter setup for the individual bead. Minimum Events: 20 count for each bead						
Use group color fo	r Chart and Allele Call Table		Lookup Table			
Prefix Group Name	Type Lookup Table	Allele Name	%Report Intensity Call Inten.	^		
A A	Other HLA A v1.10	01	50			
		02				
		04		-		
		05 Std1				
	011	Std2				
D	Other HLAD VI.10	01	50			
10		02		~		
< _			>			
Group/Allele Identifier	W of boods in this way	. 7	** Edit Bead Names	. 1		
Group Prefix:	# Of beads in this group	p. 7		-		
Group Name: A		Change Cold	r .			
Ploidy: 🔘 Dipl	oid C Haploid 🖲 Othe	er Apply this Plo	idy to all groups (loci)			
Allala Maraa			Apply to all alleles in the			
Allele Name.		Change con	same order in each group.	les		
Allele Call Parameters	for A					
C Use Relative Inter	sity for Allele call					
Reportable	Level: 25.0 % of total int	eneitu				
Intensity Th	reshold: 35 (MEI)	or only				
C http://www.						
 Intensity based A Call as this 	nere Call					
Call anythin	ig bigger main jou MPI	as an Aileite				
	Apply to	o all groups (loci)				

Figure 10.39 Parameter Setting dialog box

The highlighted lookup table is applied to the

- 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).

	1	/	iesu	115 (.05	v).		
🔳 Lookup Tal	ole Selection						
Entry Name	Last Modified	4	A	В	DR	BLD	Edit Selected Entry
(PROJECT)			HLA A Ver1.10	HLA B Ver1.10	HLA DR Ver1.10	Blood Type Ver1.00	Create New Lookup Entry
HLA Lookup Table	10/31/2003 12:56:50	PM	HLA A Ver1.10	HLA B Ver1.10	HLA DR Ver1.10	Blood Type Ver1.00	Delete Selected Entry
							Select Latest Tables
							Unselect All
							ок
							Cancel

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.

 \Rightarrow 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 **m** and **Show Type** button **1**.
 - ⇒ 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).

Click	a tab to vi	ew results	e.	Type column									
/							/	/					
***	💗 🛐 🗍	📑 🖻 📕	Å,	· · · · · · · · · · · · · · · · · · ·	ð	/							
АВ	DR BLD				/	/							
				Locus	V		A						
				Beads->	7		01	02	03	04	05	Std1	Std2
vVell Name	Sample Name	Total Events	Notes	Type(A)	Am	lv							
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 008	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 **____**.
 - \Rightarrow 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).

🍪 % А в	🕅 📗) <mark>=</mark> 3 11	ÅZ.		ð								
				Locus			A						
				Beads->			01	02	03	04	05	Std1	Std2
Vell Name	Sample Name	Total Events	Notes	Tvpe(A)	Am	lv -							
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 008	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	2.47	2.47	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	10.4	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 10.42 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.

 \Rightarrow The Allele Call window is displayed (Figure 10.43).

🔳 Allele Call -	HLA	-A_B_I	DR_BL	D ()								
BG	BG IITr III IIII IIII IIIII IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII											
		A	в	DR	BLD	^						
C [A1] blank												
☞ [B1] id0001		01 02 Std1 Std2	01 02 05 Std1 Std2	02 05 Std1 Std2	A O Std							
← [C1] id0002	2	02 03 04 05 Std1 Std2	01 02 04 05 Std1 Std2	02 05 Std1 Std2	0 Std							
← [D1] id0003	7	01 03 05 Std1 Std2	01 02 03 04 05 Std1 Std2	Std1 Std2	A Std							
C [E1] id0004		01 02 03 04 Std1 Std2	02 05 Std1 Std2	02 03 04 05 Std1 Std2	0 Std	<u>v</u>						
						1						

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.

		1		6		_	1.						
			-	Locus	<u> </u>	<u> </u>	A						
<u> </u>			-	Beads->			01	02	03	04	05	Std1	Std2
Vell Name	Sample Name	Total Events	Notes	Type(A)	Am	IV 0	ļ						
A1	blank	5200		No Matches	Ď		110	1 110		-	-	1000	1000
81	1d0001	5866	+	A 003. A 010		D°	110	110	50	3	30	1000	1000
		1000	+	4.010	-	12	22	05	54	50	249	1000	1000
	100002	4680	4	No Matches		15	442	20	445	26	125	1000	1000
54	100005	4973	+	A 007. A 005	0	12	142	1.42	143	105	10	1000	1000
E1	100004	4970	-	A 009, A 010	5	21	247	247	248	66	70	1000	1000
G1	100005	5152	+	A 005 A 010	0	17	186	186	50	24	10	1000	1000
HI	id0007	5950	-	A 009 A 010	0	12	181	181	181	54	5	1000	1000
82	100001	4845	-	A 004 A 009	5	21	257	257	69	241	256	1000	1000
B2	idnona	5591	-	A 007 A 009	0	4	11	35	133	134	142	1000	1000
C2	id0010	5016	1	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
Ty sa id Amb	pe for ample — 10001 biguity —	A 003	. A	010				1	– Do nu am	uble-o mber ıbiguit	click th to disp ty can	ne Arr play tł didate	n ne e(s).

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 **____**.
 - \Rightarrow 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.

1	o/ [<u>a</u>			2n	A		*								
88	70	• 🗉	<u> </u>	j= l	J 👖	Z*		U I								
A	в	DR	BLD													
							Locus			в						
							Beads->			01	02	03	04	05	Std1	Std2
vVell Na	ame	Sample N	lame	Total Eve	nts	Notes	Type/B)	Am	lv .							
A1		blank		5200			No Matches	0	0	-	-	-	-		-	
B1		id0001		5866			B 001. B 003	2	115	106	67	7	41	172	1000	1000
							P /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									
						$ \rangle$	B 003. B 009									
							B 007. B 009 /									
							B 004. B 009									
						V.	B 903									
C1		id0002		4680		1	B 001. B 008	9	20	178	179	44	127	183	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	228	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	1		B 001	0	5	8	124	31	13	232	1000	1000
D2		id0011		4881	1	1	B 001, B 003	2	15	111	70	7	10	170	1000	1000
E2		id0012		5216	/	1	B 005, B 010	7	17	253	254	63	176	18	1000	1000
F2		id0013		5639 /		1	B 001. B 008	9	20	263	68	17	170	177	1000	1000
				7					_							

Double-click the Iv number to display the inversion candidates

Inversion candidates for the B 001,B 003 genotype called for sample id0001.

Figure 10.45 Typing table, gradient background

The Iv column shows the number of inversion candidates for a type. Doubleclick the Iv number to view the inversion candidates for a particular type.

%	• 🖭 💷	📑 🖻 📕	Åz↓	ю	ĨĨ C	Ì								
A B	DR BLD													
				Locus				в						
				Beads-	>	<u> </u>		01	02	03 0	04	05	Std1	Std2
vVell Name	Sample Name	Total Events	Notes	Type/B	1	Am	lv							
A1	blank	5200		No Mate	hee	0	0	-	-	-	-			-
B1	id0001	5866		B 001.	B 003	2	15	106	67	7	41	172	1000	1000
			\subseteq	B 001.										
				D-007										
				B 001.	3 002			_						
				B 002.	3 007									
				B 001.	3 008									
				B 001.	3 010									
				B 007.	3 010									
<u> </u>			\square	B 007.	3 008	<u> </u>								
			H	B 009.	3 010	-	-							
<u> </u>			H	B 008.	3 009	-								
			H	B 001.	3 004	-								
<u> </u>			1	B 003.	2 009 2 009		-							
			/	B 004	3 003	-								
			ľ –	B 003	5 003	-								
C1	140002	4680		B 001	3.008	9	20	178	179	44	127	133	1000	1000
D1	id0003	4973		B 006.	3 010	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 Mate	hes	0	0	3	11	26	5	9	1000	1000
G1	id0006	5152		B 001. I	3 002	1	11	107	107	106	6	97	1000	1000
H1	id0007	5660		B 006. I	3 010	5	27	176	177	176	137	142	1000	1000
A2	id0008	4845		B 002.	3 003	1	11	228	229	57	10	17	1000	1000
B2	id0009	5591		No Mat	ches	0	2	54	15	212	11	18	1000	1000
				B 002.	3 003									
				B 002.										
C2	id0010	5016		B 001.		0	5	8	124	31	13	232	1000	1000
D2	id0011	4881		B 001. I	3 003	2	15	111	70	7	10	170	1000	1000
E2	id0012	5216		B 005.	3 010	7	17	253	254	63	176	18	1000	1000
F2	id0013	5639		B 001.	3 008	9	20	263	68	17	170	177	1000	1000
	ļ	Locus Beads->			B	02		03	04	05	Std1	Std	2	
•		Type(B)	Am	lv –										
Genot	ype	No Matches	0	0	-		-	-		-	-	-	-	
	call —	B 001 B 003	2	15	106		67	7	4	1 17	2 1	000	1000	
	Juli	D 004	-											
	/	0.007												
	//	B 007												
	B 001	is an inv	ersio	n ca	ndidat	e be	eca	use th	e aen	otype	is a r	oossih	le cal	l if

B 001, - is an inversion candidate because the genotype is a possible call if the MFI B 01 < threshold.

B 007, - $\,$ is an inversion candidate because the genotype is a possible call if the MFI B 02 is < threshold.

Figure 10.46 Typing table

CHAPTER REPORTS

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.
 - \Rightarrow 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.
 - \Rightarrow 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.



Landscape orientation selected for 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 **1** 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.
 - \Rightarrow 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).

 \Rightarrow The Preview window opens and displays the report (Figure 11.4).

			10/2	1/2002 12:3
Sample Information				
[File Information]				
<< C:\Program Files\HitachiSoft\MasterPlex G	\SampleData\Samp	ple1.csv >>		
Program: LUMINEX				
Build: VERSION 1.7.69				
Date: 5/29/02 1:23:45 PM				
Serial Number: LX1000000000				
Session: 0001-00-0123				
Operator: SAMPLE				
Heater Temparature: 55				
Number Of Samples: 72				
Minimum Events: 0				
[Reckaround Information]				
[A1] heads only: Local Background				
[P1] b409 block: Local Background				
[C1] h400 blank. Local Background				
[64] h16396 blank, Eddar Background				
(P4) h10200 blank. Eocal Dackground				
[04] h16236 blank. Local Background				
[04] h10200 blank. Eddal Dackground				
(P7) MC401 blank. Local Dackground				
[C7] h16401 blank: Local Background				
[C/] ITB401 blank. Local Background				
Lable C-I December 1				
[Allele Call Parameters]				
Parameter-Set Name:				
Bead Count Threshold: 20				
Allele Call Parameter Setting Mode: Use Group(Locu	s) Parameters.			
Prefix Locus Name Ploidy Allele Name	R!%	MH		
If If Other 73A	25.0%	35		
736				
93A				

Figure 11.4 Report Preview window

- 2. To scale the view, click the scale toolbar button $\pm 100\%$ and choose a view option.
- 3. To close the Preview window, click the Close button \times .

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 🊧.
 - \Rightarrow The Find text dialog box appears Figure 11.5.

Find text	? 🛛
Text to find	
Uptions	
	C Current page
ОК	Cancel

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 \blacksquare . \Rightarrow The Save As dialog box appears (Figure 11.6).

Save As					? 🗙
Save jn: 🔀	SampleData	•	← 🗈	ď I	
File <u>n</u> ame:					<u>S</u> ave
Save as type:	Report file (*.frp)		•		Cancel

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 B. \Rightarrow The Print dialog box appears (Figure 11.7).

Print	? 🛛
Printer Name: I I HP LaserJet 2100 Series PCL 6	Properties
Page range	Copies Number of copies:
Print All pages	OK Cancel

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.

 \Rightarrow The Preview window opens (Figure 11.8).

] #1 k? ×					
						10/21/2002
Sample Info	rmation					
[File Informa	tion]					
<< C:\F	rogram Files\Hita	chiSoft\MasterPlex G1	\SampleData\Sam	ple1.csv >>		
Program	1: LUMINEX					
Build: V	ERSION 1.7.69					
Date: 5	29/02 1:23:45 PM	1				
Serial N	umber: LX100000	00000				
Session	: 0001-00-0123					
Operato	r: SAMPLE					
Heater	Cemparature: 55					
Number	Of Samples: 72					
Minimu	m Events: 0					
[Background	Information]					
[A1] be:	ads only: Local B	ackground				
[B1] h4	8 blank: Local B	ackground				
[C1] h4	8 blank: Local B	ackground				
[A4] h18	5236 blank: Local	Background				
[B4] h1	5236 blank: Local	Background				
[C4] h18	5236 blank: Local	Background				
[A7] h1	5401 blank: Local	Background				
[B7] h18	6401 blank: Local	Background				
[C7] h1	5401 blank: Local	Background				
[Allele Call F	arameters]					
Parameter-Se	t Name:					
Bead Count 1	hreshold: 20					
Allele Call Pa	rameter Setting N	Inde: Use Group(Locu	s) Parameters.			
Prefix	.ocus Name P	oidy Allele Name	RI%	MEL		
II.	r o	ther 73A	25.0%	35	_	
		73G				
		93A				
		930				

Figure 11.8 Preview window

Click the Open Report toolbar button [→].
 ⇒ The Open dialog box appears (Figure 11.9).

Open					? 🗙
Look in: 🗀	SampleData	•	← 🗈	💣 🎫-	
File <u>n</u> ame:				<u>0</u> pe	n
Files of <u>t</u> ype:	Report file (*.frp)		•	Cano	el

Figure 11.9 Open dialog box

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

APPENDIX 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

- To view the general options, select Options → Set Application Options from the menu bar.
 - \Rightarrow The Application Options dialog box appears (Figure A.1).

Application Options	
General Background Clustering Tool Plugins	
Bead Name Style	
Locus Name + Allele Name Original Read Name	
Start Up Window After Data Loading	
Show Table View	
C Show Graph View	
Table View Gradation Background	
Use Allele Call Color for gradation background	
Group Color #1 Change Color	
Group Color #2 Change Color	
Heatmap Options	
Heatmap Bar Size: 3 🗣 Pixel(s) / Allele	
OK Reset All Cancel	1
	J

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).

				LOCU	is nan	ne (firs	st row) and	allele	name	es (ne	xt row	!)
			/	1									
			Locus /	SNP1	1	SNP2		SNP3		SNP4		SNP5	
			Beads->	wt	mt	wt	mt	wt	mt	wt	mt	wt	mt
vVell 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

	1	1											
			Locus										
			Beads->	SNP1 wt	SNP1 mt	SNP2 wt	SNP2 mt	SNP3 wt	SNP3 mt	SNP4 wt	SNP4 mt	SNP5 wt	SNP5 mt
vVell 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
84	no dos	24		-	-	-	-	-	-	-		-	-

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.

	T C G C G 433 35 476 117 35 444 34 491 116 33 459 35 508 125 38 450 10 230 125 33	C 433	Y 273	х	Т	с	Beads->			
Vell Name Samole Name Total Events Notes Notes	433 35 476 117 35 444 34 491 116 33 458 35 506 125 33	433	273							
11 om55 2a 2000 15 16 955 723 433 947 111 111 0m55 2b 2000 14 315 414 221 443 94 415 111 111 0m55 2b 2000 15 327 443 244 94 415 111 121 om55 2c 2000 15 327 447 285 453 95 598 122 0m37 4b 2000 15 327 447 245 447 44 244 447 44 316 144 22 0m37 4b 2000 110 000 268 441 248 446 128 22 vm32 5b 2000 174 221 446 247 448 188 12 vm32 5b 2000 174 221 446 248 68 598 128 133 0m38 6b 2000 124	433 35 476 117 35 444 34 491 116 33 458 35 508 125 33 459 42 508 125 33	433	273				Notes	Total Events	Sample Name	Vell Name
11 cm5 3b 2000 14 118 414 211 444 243 251 144 243 251 144 243 251 144 243 251 144 243 251 144 243 251 144 243 255 265 122 cm37 4b 2000 19 282 447 242 451 447 42 516 144 22 cm37 4b 2000 111 301 463 224 447 42 516 144 22 cm37 4c 2000 116 301 469 229 463 407 42 516 142 24 vin32 5c 2000 116 224 447 42 516 152 33 cm39 5c 2000 112 225 301 442 447 122 33 cm39 5c 2000 112 228 372 231 443	444 34 491 116 33 458 35 508 125 33 450 40 470 106 27			385		15		2000	cm55 2a	51
and em5 20 2000 10 222 423 285 439 596 112 cm37 4b 2000 11 324 443 286 439 449 42 516 140 cm37 4b 2000 11 324 446 284 437 42 516 140 c22 em37 4c 2000 111 301 468 284 437 42 516 140 c22 em37 4c 2000 110 409 428 444 98 444 188 122 win32 5c 2000 174 271 446 287 514 69 595 128 33 em39 8c 2000 174 221 446 287 514 69 595 128 33 em39 8c 2000 172 285 435 291 443 44 124 23 em39 8c 2000 1124	458 35 508 125 33	444	271	414	315	14		2000	cm55 2b	-1
C2 cm37 4a 2000 15 28 447 28 487 448 476 15 128 20 cm37 4b 2000 28 448 648 284 448 42 42 55 144 22 cm57 4c 2000 11 001 489 284 448 28 448 438 46 434 45 55 144 22 vm52 5b 2000 116 269 447 291 438 46 434 138 23 cm39 5c 2000 116 261 445 297 543 46 585 132 23 cm39 5c 2000 168 281 481 30 442 441 124 33 cm39 5c 2000 112 226 398 283 414 44 447 114 21 wm32 5c 2000 114 224 378 288 <td>450 40 470 105 07</td> <td>458</td> <td>285</td> <td>423</td> <td>327</td> <td>13</td> <td></td> <td>2000</td> <td>cm55 2c</td> <td>31</td>	450 40 470 105 07	458	285	423	327	13		2000	cm55 2c	31
22 cm37 4b 2000 92 241 464 924 447 42 516 144 22 cm37 4b 2000 111 301 403 284 437 443 84 432 516 144 22 cm37 4b 2000 116 268 447 291 443 46 452 136 32 cm39 5b 2000 174 271 445 297 514 50 516 152 33 cm39 5b 2000 174 271 445 297 514 50 516 152 33 cm39 5b 2000 188 281 432 68 501 124 22 vin32 5b 2000 1108 228 392 294 441 424 431 110 31 bm33 1b 2000 1168 228 397 291 435 342 200 11	400 40 478 120 37	450	296	447	328	15		2000	cm37 4a	22
22 cm37 4_c 2000 11 801 409 269 443 86 462 172 20 vm32 5c 2000 160 268 441 281 446 982 443 46 473 184 46 474 184 42 vm32 5c 2000 174 271 445 297 514 50 516 183 33 cm39 6c 2000 1215 265 461 901 432 65 505 182 33 cm39 6c 2000 124 225 381 201 443 44 441 124 22 vm32 5c 2000 102 228 382 221 215 461 431 424 474 124 22 vm32 5c 2000 102 228 382 228 441 43 42 201 11 bm33 1a 2000 1141 241	487 42 516 140 128	487	294	464	341	32		2000	cm37 4b	02
22 win32 5b 2000 160 288 417 291 438 46 454 158 42 win32 5c 2000 174 271 448 297 514 50 516 183 523 cm39 5c 2000 215 255 481 901 492 65 516 183 33 cm39 5c 2000 168 281 435 281 414 42 481 122 33 cm39 5b 2000 124 226 392 281 414 42 441 124 22 win32 5a 2000 114 226 392 281 411 42 441 114 21 bm33 1a 2000 1141 241 372 281 435 35 12 108 21 bm33 1a 2000 1166 270 443 276 445 38 427 108	464 38 464 122 36	464	269	409		11		2000	cm37 4c	2
vmm32 5c. 2000 174 271 446 287 514 60 516 173 cm39 8c. 2000 215 285 446 287 514 68 595 128 x3 cm39 8c. 2000 215 285 445 291 442 68 595 128 x3 cm39 8b 2000 104 226 938 291 444 42 481 122 vm32 5c 2000 104 228 938 292 441 447 447 116 11 bm33 1b 2000 1141 241 974 268 431 33 12 291 12 bm33 1b 2000 1168 220 443 276 445 34 200 14 844 974 248 443 124 200 144 34 276 448 200 10 90 90 90 <	493 46 494 136 312	493	291	417	268	160		2000	wim32.5b	32
33 cm39 & 6c 2000 215 285 481 301 422 683 505 1128 x3.0 cm39 & 6a 2000 168 281 455 291 454 42 481 120 x3.0 cm39 & 6a 2000 1168 281 455 291 454 42 481 120 x3.0 cm39 & 6b 2000 1108 228 372 291 455 447 112 x2 vin32 & 5a 2000 1108 228 372 291 455 447 112 x1 bm33 & 1a 2000 1156 263 385 283 441 35 12 393 x1 bm33 & 1a 2000 1166 270 445 276 448 35 142 280 x1 bm33 & 1a 2000 1166 270 448 276 448 186 200 x1 bm33 & 1a	514 50 516 132 315	514	297	446	271	174		2000	wim32.5c	+12
A3 cm39.8a 2000 168 281 435 291 448 42 481 172 33 cm39.8a 2000 124 226 389 291 448 42 481 172 22 win32.5a 2000 103 228 372 291 435 40 447 124 22 win32.5a 2000 1103 228 372 291 435 40 447 124 21 bm33.1a 2000 1141 241 374 268 443 33 12 283 21 bm33.1b 2000 156 263 445 35 448 82 200 11 bm44.3a 2000 156 220 448 247 448 18 82 200 18 200 18 200 18 200 18 200 18 200 200 200 200 200 20	492 63 505 128 302	492	301	481	285	215		2000	cm39.6c	3
B3 cm39 8b 2000 124 226 998 293 411 4 474 1124 22 wm32 5a 2000 100 228 978 281 435 40 4471 110 91 bm33 1a 2000 141 241 974 282 435 40 4471 110 91 bm33 1b 2000 141 241 974 282 435 42 200 91 bm33 1c 2000 156 270 443 276 443 92 16 200 911 bm44 3a 2000 159 220 442 276 443 92 16 200 911 bm44 3a 2000 159 220 443 276 443 92 16 93 92 bm44 3b 2000 159 289 427 447 10 19 19 19 19 19 15	484 42 481 120 292	484	291	455	281	163		2000	cm39.6a	43
vvm32 5a 2000 100 228 972 251 425 40 447 111 bm33 1a 2000 141 241 974 259 435 35 12 198 21 bm33 1a 2000 156 263 385 283 445 35 12 198 21 bm33 1c 2000 156 263 385 283 445 35 442 200 11 bm43 3a 2000 156 283 436 276 448 276 448 210 16 200 15 199 199 199 199 199 199 199 199 199 199 199 199 199 199 199 199 199 199 199 199 199 199 199 199 199 199 199 199 199 199 199 199 199 199 199 199	471 43 474 124 284	47.1	289	398	226	124		2000	cm39.6b	33
B1 bm3 1a 2000 141 241 974 268 481 95 12 1983 C1 bm3 1b 2000 156 263 963 263 425 95 442 90 425 95 442 90 425 95 442 90 445 95 442 90 445 92 18 2000 156 270 443 276 446 92 18 2000 155 263 447 41 19 195 195 444 44 19 195 195 444 42 200 15 15 16 16 270 443 240 441 19 195 155 15 15 15 15 15 15 15 15 15 15 15 15 15 15 15 15 15 15 15 15 15 15 16 15 16 15	455 40 447 110 307	455	291	372	228	103		2000	wim32.5a	2
C1 bm331b 2000 156 263 365 268 445 35 42 200 01 bm331c 2000 166 270 449 276 448 32 16 200 11 bm443a 2000 155 288 427 288 447 34 19 19 19 42 bm443b 2000 138 239 584 278 447 32 15 19 19 19 19 19 19 19 19 19 19 19 19 19 19 19 19 19 19 19 19 19 19 19 19 19 19 19 19 19 19 19 19 19 19 19 19 19 19 19 19 19 19 19 19 19 19 19 19 19 19 19 19	401 35 12 194 295	401	268	374	241	141		2000	bm331a	31
bm31c 2000 166 270 443 276 443 2 16 800 11 bm443a 2000 155 283 427 260 420 41 19 195 195 195 195 195 195 195 195 195 195 195 195 195 195 195 195 195 195 195 195 195 195 195 195 195 195 195 195 195 195 195 195 195 195 195 195 195 195 195 195 195 195 195 195 195 195 195 195 195 195 195 195 195 195 195 195 195 195 195 195 195 195 195 195 195 195 195 195 195 195 195 195 195 195 195 195	445 35 42 207 289	445	283	385	263	156		2000	bm331b	21
H1 bm44 3a 2000 159 283 427 286 470 41 19 195 k2 bm44 3b 2000 198 280 384 273 427 32 115 191	446 32 18 207 301	446	276	449	270	166		2000	bm331c	21
42 bm44 3b 2000 136 230 384 273 437 32 15 191	470 41 19 195 286	470	288	427	283	159		2000	bm44 3a	-11
101 070 071 070 011 11	437 32 15 191 294	437	273	384	230	136		2000	bm44 3b	42
32 bm44 3c 2000 131 241 370 271 425 24 11 130	425 24 11 190 275	425	271	370	241	131		2000	bm44 3c	32
A1 ren 2000		-	-	-	-	-		2000	rcn	A1
03 ren 2000		-	-	-	-	-		2000	rcn	03
			1		1	1				

∠ Locus (group) names (first row) allele names (next row)

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).
 - \Rightarrow 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**.

 \Rightarrow 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.

 \Rightarrow 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).

Application Options
General Background Clustering Tool Plugins
Bead Name Style
C Locue Name + Allele Name
C Original Bead Name
Start Up Window After Data Loading
Show Table View
C Show Graph View
Table View Gradation Rackground
Use Allele Call Color for gradation background
Group Color #1 Change Color
Group Color #2 Change Color
Heatmap Options
Heatmap Bar Size: 3 🕒 Pixel(s) / Allele
OK Reset All Cancel

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 MasterPlexTM 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** \rightarrow **Application Options** from the menu bar. \Rightarrow The Application Options dialog box opens (Figure A.8).

Application Options	
General Background Clustering Tool	Plugins
Automatic Background Sam	Die Recognition
Perform Automatic Background	Sample Detection on data load
Background Keywords	
bead only	Add New Keyword
beads only bkg	Delete Selected Keyword
background	
No. IN	
NOTE: These parameters are effective	only to new projects.
	set All Cancel

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).
 - \Rightarrow 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).

Add a Background K	eyword	×
Input a new keyword for	background	
ок	Cancel	

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.



Figure A.10 Clustering Tool window

🖲 Genotype 🛛 Expression

1. Select **Option Set** \rightarrow **Application Options** from the menu bar. \Rightarrow 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 ${}^{_{\rm TM}}$ GT software.

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

Application Opt	ions			K
General Background	Clustering Tool	Plugins		
Plugins Selection	nn			
T lagins selection				
OK	R	eset All	Cancel	

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 **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).

💷 New Project Default Parameters
Allele Call Params Table View Graph View Allele Call View Cluster Analysis
Allele Call General Options
Parameter setup for the individual bead.
Use group color for Chart and Allele Call Table
Minimum Events: 20 🚖 count for each bead
Ploidy: C Diploid C Haploid 📀 Other
Allele Call Parameters
G. Use Belative Intensity for Allele call
Benotable Level: 25 🗣 % of total intensity
Intensity Threshold: 35 Image: (MFI)
C. Ister At the Cell
Call anothing bigger than 50
NOTE: These parameters are effective only to new projects.
OK Reset All Cancel

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

Use group color for	Choose this option to display the group colors
Chart and Allele	(specified in the Parameter Settings dialog box) in
Call table	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 The software calls the allele if all of the following for Allele Call conditions are met:

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

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

🔳 Par	ameter S	etting						
Group s	et: Save se	etting as	mport Setting	•			Cancel	ок
IV Para IV Use	ameter setu; group color	o for the individ for Chart and	ual bead. Allele Call Table	Minimum Event	s: 20	count for	each bead Lookup Tab	le
Prefix	Group Nar	ne Type	Lookup Table	Allele Name	%Report	able Level	Intensity Threshold	Call Intensity
IA	IA	Other						
ID.		Olle au		16124C 16126C 16129A Anderson	25.0% 25.0% 25.0% 25.0%		35 35 35 35	
16		Uther		16217C 16223T 16224C Anderson	25.0% 25.0% 25.0% 25.0%		35 35 35 35	
IC1	IC1	Other		16292T 16 16294T	25.0% 25.0%		35 35	v
-Group//	Allele Identifi	er						
Group I	Prefix: IA	#ofb	eads in this gro	up: 4				* Eart Bead Names
Group I	Name: 🛛		_	Cha	inge Color			
F	Ploidy: O D	iploid C I	Haploid 🕥 Otl	ner App	y this Ploid	y to all grou	ips (loci)	
Allele N	lame: 1613	24C		Cha	inge Color	App sam	ily to all alleles in the e order in each grou ily to all same name :	p. alleles
-Allele C	all Paramete e Relative In Reportak Intensity ensity basec Call anyt	rs for IA 16124 tensity for Allel le Level: 25 Threshold: 35 I Allele Call hing bigger tha	e call 0 % of total in (MFI)	itensity Fl as an Allele				
			Ap	ply to all beads	;			

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).

📗 Typing	- Sample2 (Samp	le2.gtp)										- 0	x
%	• 🛛 🥅	<u>-</u> 3 📶	24 m	0									
			Locus	IA.				B				IC1	^
			Beads->	16124C	16126C	16129A	Anderson	16217C	16223T	16224C	Anderson	16292T 1I	
vVell 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	v

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

			Locus	IA				IB			
			Beads->	16124C	16126C	16129A	Andersor	16217C	16223T	16224C	Anderson
v/vell 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
	1	1	1								

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).

Allele Call - Sample2 (Sample2.gtp) 												
BG 🎫 💶 🕸 🚺	AT	×										
Allele Call Allele Frequency	y Ge	enotype Frequ	ency Haple	type Frequer	ncy							
		IA	IB	IC1	IC2	ID	IIA1	IIA2	IIB	IIC	IID	^
🖲 [F1] 48-1		16126C	Anderson		j.							
C [F2] 48-1		16126C	Anderson									
⊂ [F3] 48-1d	~	16126C	Anderson									
C [A1] beads only new												
[B4] beads new												
C [C4] beadsold												
C [B2] 47-1	•	Anderson	Anderson									
C [B3] 47-1d	~	Anderson	Anderson									
C [B1] 47-1	~	Anderson	16217C Anderson									
○ [C1] 47-2	~		Anderson	Anderson	16311C 16311C 16320T	16362T						
C [C2] 47-2	~		Anderson	Anderson	16311C 16311C 16320T	16362T						
⊂ [C3] 47-2d	~		Anderson	Anderson	16311C 16311C 16320T	16362T						
C [G1] 48-2	~		Anderson	16294T	Anderson	16362T						-
C [G2] 48-2	~		Anderson	16294T	Anderson	16362T						
C [G3] 48-2d	~		Anderson	16294T	Anderson	16362T						
C [E1] 47-4	~									195C	263G	
C [A2] 48-4	~									195C	263G	
C [E2] 47-4	~									195C	263G	
C [A3] 48-4	~									195C	263G	
○ [E3] 47-4d	~									195C	263G	
C [A4] 48-4d	~									195C	263G	~
<											1	8
										_		

🔳 Allele Call - Sample	e2 (S	ample2.gt	p)							X
BG 🏭 III 🌸 🙀	ê <mark>.</mark>	- x								
Allele Call Allele Frequency	Ge	notype Frequ	ency Haplotype Fre	auency						
[······,	1	10	тр	101	102	ID	TTAI	1142	TTP	
@ [F1] 48-1	17-	161260	10 Anderson	101	102	10	1141	1142	110	- 11
C [F2] 48-1	7	161260	Anderson							1
C [F3] 48-1d	7	161260	Anderson							-
C [A1] beads only new		101200	Anderson							-
C [B4] beads new										-
C [C4] beadsold							-			-
C [B2] 47-1	7	Anderson	Anderson							
C [B3] 47-1d	7	Anderson	Anderson							-
C [B1] 47-1	~	Anderson	16217C, Anderson							
C [C1] 47-2	~		Anderson	Anderson	16311C,16311C 16320T	16362T				
C [C2] 47-2	~		Anderson	Anderson	16311C,16311C 16320T	16362T				-
C [C3] 47-2d	~		Anderson	Anderson	16311C,16311C 16320T	16362T				-
C [G1] 48-2	~		Anderson	16294T	Anderson	16362T				
C [G2] 48-2	~		Anderson	16294T	Anderson	16362T				
C [G3] 48-2d	~		Anderson	16294T	Anderson	16362T				
C [E1] 47-4	~									
C [A2] 48-4	~									
C [E2] 47-4	~									
C [A3] 48-4	~									
C [E3] 47-4d	~									
C [A4] 48-4d	~									
C [D1] 47-3	~						73A	93G	Anderson	
C [D2] 47-3	~						73A	93G	Anderson	~
									3	-

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	Choose this option to paint (highlight)
genotype is same as the	alleles in the Allele Call table that have the
reference	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).

Allele Frequer	cy Ge	enotype Frequ	ency Haplotype Fre	quency						
		IA	IB	IC1	IC2	ID	IIA1	IIA2	IIB	Т
¥ [C1] 47-2	~	-j	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	
C [D2] 47-3	~				,		73A	93G	Anderson	
C [D3] 47-3d	~						73A	93G	Anderson	
[D1] 47-3	~				,		73A	93G	Anderson	
[C4] beadsold										
[E2] 47-4	~									
E1] 47-4	~				,					
C [A2] 48-4	~									

💶 Allele Call - SampleSmall 📃 🗖 🔀											
III 🗰 🏭 🗙											
Allele Call Allele Frequency Genotype Frequency Haplotype Frequency											
		mt	SNP2	SNP3	SNP4	SNP5	SNP6	SNP7	SNP8	SNP9	SNP10
• [A2] 8		wt	wt	wt	wt	wt	wt	wt	wt	wt	wt
C [H1] 7	•	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt
C [B1] 1	•	wt	wt	wt	wt, <mark>mt</mark>	wt	wt	wt	wt	wt	wt
C [B2] 9	•	wt	wt	wt	wt, <mark>mt</mark>	wt	wt	wt	wt	wt	wt
C [F1] 5	•	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt
C [G1] 6	•	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt
C [B3] 10	•	wt	wt	wt	wt, mt	wt	wt	wt	wt	wt	wt
C [C1] 2	•	wt	wt,mt	wt	wt	wt	wt	wt	wt	wt	wt
C [E1] 4	~	wt	wt	wt	wt	mt	mt	wt	wt	wt	wt
C [D1] 3	~	wt	wt,mt	mt	wt, mt	mt	mt	wt	wt	wt	wt
🖸 [A1] no dna	~										
<											>
	_										

Figure B.13 Allele Call table

Same genotypes painted (top), different genotypes painted (bottom)

Allele Call Table Options

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

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

		A	lele Call -	Sam	pleSm	all (Sa	mpleSr	nall.gtj	p)						×
	В	a	II III 🎕		AT AT		×								
	Allele Call Allele Frequency Genotype Frequency Haplotype Frequency														
	6	\mathbf{r}			SNP1	SNP2	SNP3	SNP4	SNP5	SNP6	SNP7	SNP8	SNP9	SNP10	^
	۹	ſ	1]2		wt	wt mt	wt wt								
Radio buttons for	C	[8	1]1	~	wt	wt wt									
selecting a reference	C	[A	2]8	~	wt	wt wt									
sample are available	C	[F	1]5	~	wt	wt wt									
	C	[2] 9	~	wt	wt wt	wt wt	wt mt	wt wt	wt wt	wt wt	wt wt	wt wt	wt wt	
	S	/th	1]7	~	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	~
	-														

Allele Call	- Sa	ampleS	mall (S	iample	Small.	gtp)						X
BG III ITT 🐉 🗱 🚛 🗙												
Allele Call Allele Frequency Genotype Frequency Haplotype Frequency												
		SNP1	SNP2	SNP3	SNP4	SNP5	SNP6	SNP7	SNP8	SNP9	SNP10	^
[C1] 2	~	wt	wt mt	wt wt								
[81] 1	~	wt	wt wt	wt wt	wt wt	wt wt	wt wt	wt wt	wt wt	wt wt	wt wt	
[A2] 8	~	wt	wt wt	wt wt	wt wt	wt wt	wt wt	wt wt	wt wt	wt wt	wt	
[F1] 5	~	wt	<mark>wt</mark> wt	wt wt	wt							
[82] 9	~	wt	<mark>wt</mark> wt	wt wt	wt mt	wt wt	wt wt	wt wt	wt wt	wt wt	wt wt	
[H1] 7	~	wt	<mark>wt</mark> wt	wt wt	wt	~						

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.

💷 New Project Default Parameters								
Allele Call Params Table View Graph View Allele Call View Cluster Analysis								
Default Cluster Analysis Method								
Ward's Method								
Parameter for Flexible Method: 0.25								
Default Cluster Analysis Options								
C Cluster Analysis By Expression								
 Cluster Analysis By Genotype/Haplotype 								
OK Reset All Cancel								

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**.

APPENDIX TOOLBARS

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	D	Displays the Open dialog box so that a Luminex [®] results file (.csv) may be opened.
File → Open Project File	à	Displays the Open dialog box so that a project (.gtp) may be opened.
File → Save Project	H	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	4	Displays the Allele Call table for the active results.
	Ö	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	g table toolbal 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.
1	Displays the background-adjusted MFI data for the alleles in the Typing table.
Ÿ	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 <i>striped</i> 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.
<u>ب</u>	Displays the dendrogram.
	Displays the Multi Graph view for the active results.
₽	Sorts the samples in the Typing table by

Table C 2 Typing table toolbar buttons and functions

	results.
₽↓	Sorts the samples in the Typing table by homology to the expression levels of a user- selected sample.

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

Table C.2 Typing table toolbar buttons and functions

C.3 Multi Graph View Toolbar

۲	G 🗄 📕	🕼	B ABY 👼	$M_{\rm F_{\rm I}}$ RI	Sz.	1 3 1		H x1.0	V x1.0
---	-------	---	---------	------------------------	-----	-------	--	--------	--------

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.
4	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.
LØ	Paints only the called alleles in the Multi
	Compare and Depth bar graphs.
ABC	Displays the x-axis labels vertically in the Multi
	Compare graph, Depth graph, and Threshold
	editing tab.

Multi Graph View Toolbar Button	Function
ABC	Includes the group and allele name in the x-
	axis label of the Multi Compare graph, Depth
	Braph, and Threshold editing tab.
<u>o040</u>	Displays a subset of the x-axis labels so that
	Compare graph Depth graph and Threshold
	editing tab.
Me	Plots the background-adjusted MFI data in the
. LI	Multi Compare and Depth bar graphs.
PT	Plots the percent relative intensity data in the
KT.	Multi Compare and Depth bar graphs.
<u>&</u>	Puts the Multi Graph view in Two Sample
ĸ	Comparison mode that enables you to plot a
	Sample by Sample scatter graph.
	Hides or unhides the Heat map in the Multi
	Graph view.
2	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
7	name tags in the Multi Compare graph.
H x 1.0	Displays a drop-down list of size options for
	the horizontal dimension of the bars in the
	Multi Compare and Depth graphs.
V x1.0	Displays a drop-down list of size options for
	the vertical dimension of the bars in the Multi
	Compare and Depth graphs.

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