ChimeRMarker™
Automated Chimerism Analysis Software

Automated Chimerism STR Analysis and Monitoring Software

- **Significantly Reduces Analysis Time... up to 85%**

- **Validated**

- **Single and Double Donor Capabilities**

- **No Data Transfer**

- **Longitudinal Monitoring**

- **Compatible with all Major Capillary Electrophoresis Systems and Chemistries**

Automated Maternal Cell Contamination (MCC) Analysis

SOFTGENETICS®

# ChimerMarker™ Chimerism Genotyping Analysis and Monitoring Software – Reduces Analysis Time…. up to 85%‡

ChimerMarker™ software, developed in collaboration with Dr. Donald Kristt*, and validated† by laboratories in the U.S. and Europe, combines accurate size and allele calls (genotyping) with automated chimerism calculations and long term monitoring. The program integrates speed and accuracy with a biologist-friendly interface. Initial analysis is typically three steps to completion:

- **GENOTYPING donor(s) and recipient**
- **AUTOMATED DETECTION of INFORMATIVE LOCI and MIXTURE DECONVOLUTION**
- **LINKED NAVIGATION directly to CHIMERISM CALCULATIONS, REPORTS and LONGITUDINAL MONITORING GRAPH**

ChimerMarker is compatible with ABI®PRISM, Beckman-CEQ™, and MegaBACE® genetic analyzers, custom primers or commercially available human identification chemistries for STR genotyping, including PowerPlex®16, PowerPlex®18D, PowerPlex®21 and PowerPlex®ESI/ESX.

ChimerMarker utilizes highly accurate size calibration and allele calling algorithms with the ability to differentiate between donor, recipient, and shared peaks in the electropherogram using the unique chimerism panel for that patient. In addition, it automatically calculates percent chimerism and quality metrics for each sample without the need to transfer data between software. ChimerMarker software can be used to monitor chimerism level in both allogeneic and autologous SCT, HSCT, BMT, post bone marrow engraftment, and PBSCT samples.

Peak Differentiation and Labeling – Immediate Visualization of Donor and Recipient Alleles in Post-Transplant Sample Electropherograms
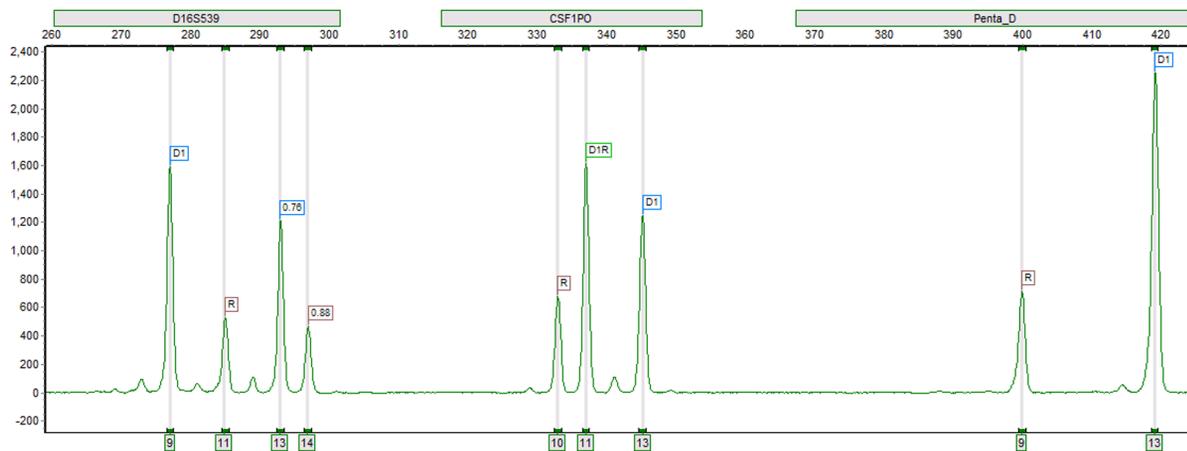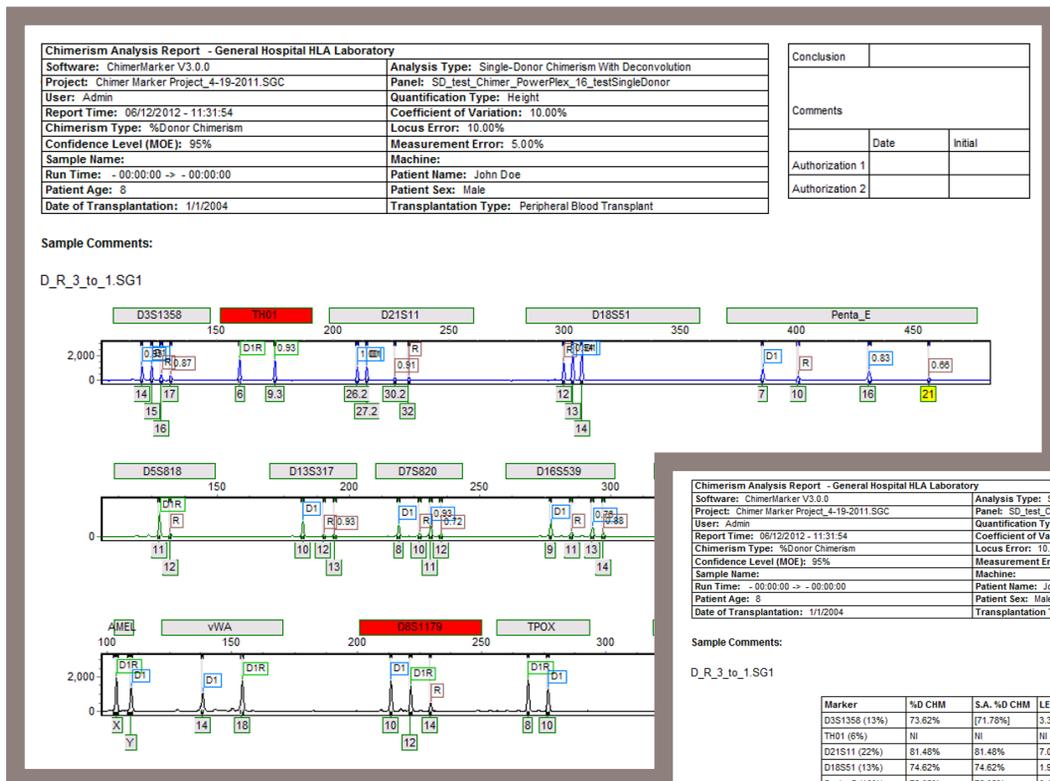

Post-Transplant Sample amplified with PowerPlex®16: ChimerMarker labels peak origin in post-transplant samples - Donor (D), Recipient (R), or D1R (Donor 1 and Recipient) for shared alleles in each locus. Heterozygous Imbalances are calculated for sister alleles of the same locus separately for donor and recipient; detecting variation from PCR and CE artifacts.

Automates Chimerism Calculations for single or double donor cases - chimerism analysis is linked to allele calling, removing the error-prone step of data transfer from genotyping software to chimerism analysis software. Results for the selected sample are displayed in a report table (center) and electropherograms (right). Non-informative loci are highlighted in yellow in the report table and red in the marker label of the electropherogram. **Non-informative** loci are excluded from the calculations. Manually excluding loci is under user management control for authorized users. Report tables and comments can be exported as .txt or .xls files, or saved and printed as image files.
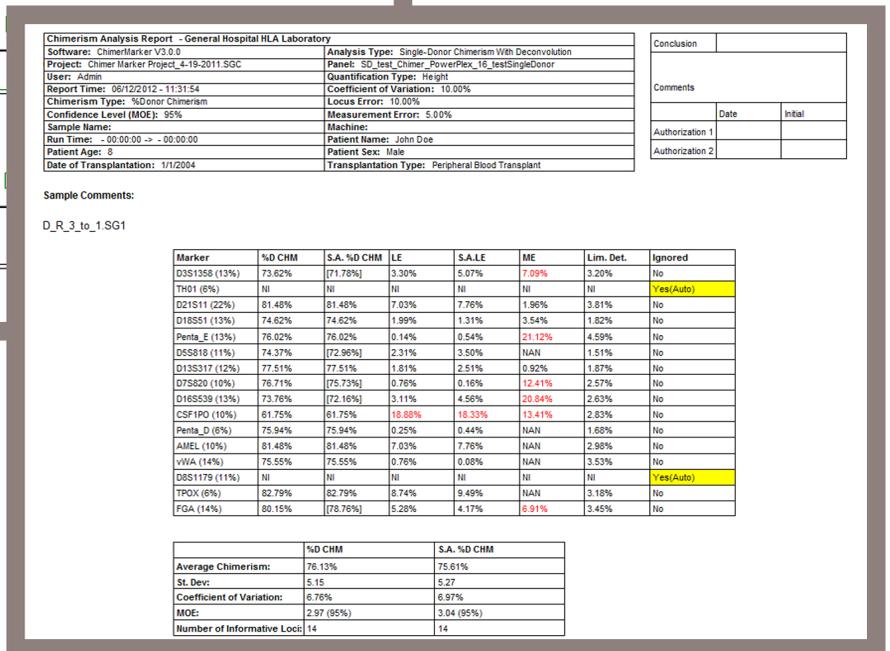
Authorized users set analysis parameters to calculate percent donor or percent recipient (%D or %R CHM), use peak height or area, Locus Error (LE), Measurement Error (ME), Coefficient of Variation, Limit of Detection, and whether or not to subtract stutter values from peaks in stutter positions (S.A.%CHM).
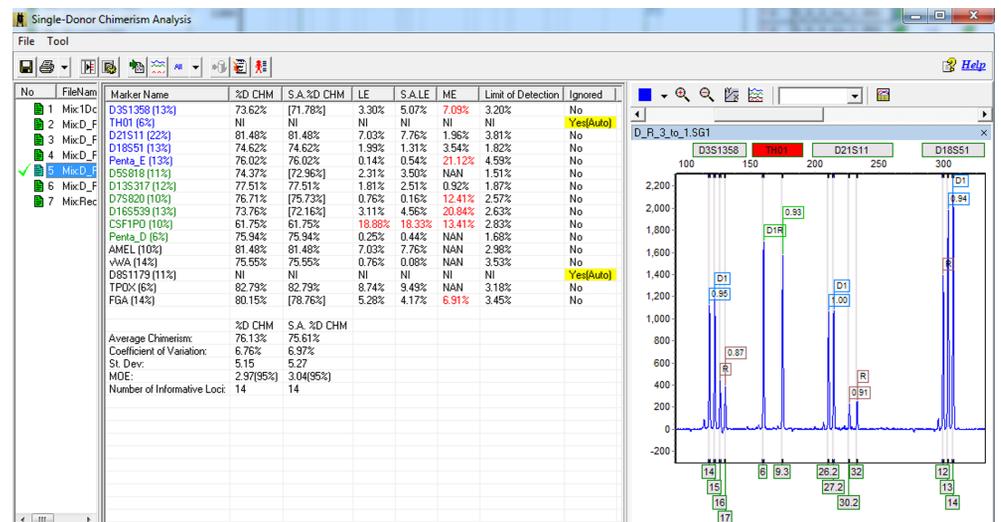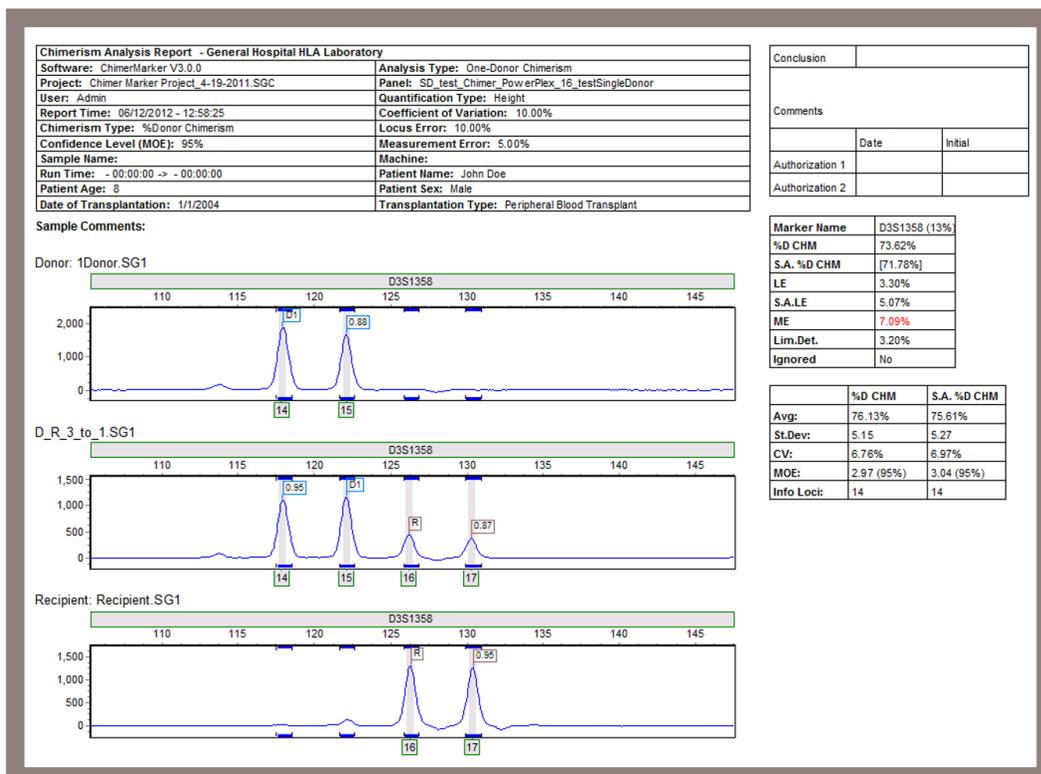
Comprehensive Reports – Single time point analysis reports contain a header with user/institution ID, patient information and analysis parameters, an authorization box for initials/date. Customize the report by selecting from a variety of options. Two popular options are pictured here. Reports may be printed directly, or saved as an image file for electronic records.

First Report (page 1)

Chimerism Analysis Report - General Hospital HLA Laboratory		
Software: ChimerMarker V3.0.0	**Analysis Type:** Single-Donor Chimerism With Deconvolution	**Conclusion**
Project: Chimer Marker Project_4-19-2011.SGC	**Panel:** SD_test_Chimer_PowerPlex_16_testSingleDonor	
User: Admin	**Quantification Type:** Height	
Report Time: 06/12/2012 - 11:31:54	**Coefficient of Variation:** 10.00%	**Comments**
Chimerism Type: %Donor Chimerism	**Locus Error:** 10.00%	
Confidence Level (MOE): 95%	**Measurement Error:** 5.00%	Date / Initial
Sample Name:	**Machine:**	**Authorization 1**
Run Time: - 00:00:00 -> - 00:00:00	**Patient Name:** John Doe	**Authorization 2**
Patient Age: 8	**Patient Sex:** Male	
Date of Transplantation: 1/1/2004	**Transplantation Type:** Peripheral Blood Transplant	

Sample Comments:

D_R_3_to_1.SG1

Complete electropherograms of the post-transplant sample with the chimerism report on the second page

Second Report (page 2)

Chimerism Analysis Report - General Hospital HLA Laboratory		
Software: ChimerMarker V3.0.0	**Analysis Type:** Single-Donor Chimerism With Deconvolution	**Conclusion**
Project: Chimer Marker Project_4-19-2011.SGC	**Panel:** SD_test_Chimer_PowerPlex_16_testSingleDonor	
User: Admin	**Quantification Type:** Height	
Report Time: 06/12/2012 - 11:31:54	**Coefficient of Variation:** 10.00%	**Comments**
Chimerism Type: %Donor Chimerism	**Locus Error:** 10.00%	
Confidence Level (MOE): 95%	**Measurement Error:** 5.00%	Date / Initial
Sample Name:	**Machine:**	**Authorization 1**
Run Time: - 00:00:00 -> - 00:00:00	**Patient Name:** John Doe	**Authorization 2**
Patient Age: 8	**Patient Sex:** Male	
Date of Transplantation: 1/1/2004	**Transplantation Type:** Peripheral Blood Transplant	

Sample Comments:

D_R_3_to_1.SG1

Marker	%D CHM	S.A. %D CHM	LE	S.A.LE	ME	Lim. Det.	Ignored
D3S1358 (13%)	73.62%	[71.78%]	3.30%	5.07%	7.09%	3.20%	No
TH01 (6%)	NI	NI	NI	NI	NI	NI	Yes(Auto)
D21S11 (22%)	81.48%	81.48%	7.03%	7.76%	1.96%	3.81%	No
D18S51 (13%)	74.62%	74.62%	1.99%	1.31%	3.54%	1.82%	No
Penta_E (13%)	76.02%	76.02%	0.14%	0.54%	21.12%	4.59%	No
D5S818 (11%)	74.37%	[72.96%]	2.31%	3.50%	NAN	1.51%	No
D13S317 (12%)	77.51%	77.51%	1.81%	2.51%	0.92%	1.87%	No
D7S820 (10%)	76.71%	[75.73%]	0.76%	0.16%	12.41%	2.57%	No
D16S539 (13%)	73.76%	[72.16%]	3.11%	4.56%	20.84%	2.63%	No
CSF1PO (10%)	61.75%	61.75%	18.88%	18.33%	13.41%	2.83%	No
Penta_D (6%)	75.94%	75.94%	0.25%	0.44%	NAN	1.68%	No
AMEL (10%)	81.48%	81.48%	7.03%	7.76%	NAN	2.98%	No
vWA (14%)	75.55%	75.55%	0.76%	0.08%	NAN	3.53%	No
D8S1179 (11%)	NI	NI	NI	NI	NI	NI	Yes(Auto)
TPOX (6%)	82.79%	82.79%	8.74%	9.49%	NAN	3.18%	No
FGA (14%)	80.15%	[78.76%]	5.28%	4.17%	6.91%	3.45%	No

	%D CHM	S.A. %D CHM
Average Chimerism:	76.13%	75.61%
St. Dev:	5.15	5.27
Coefficient of Variation:	6.76%	6.97%
MOE:	2.97 (95%)	3.04 (95%)
Number of Informative Loci:	14	14

Third Report

Chimerism Analysis Report - General Hospital HLA Laboratory		
Software: ChimerMarker V3.0.0	**Analysis Type:** One-Donor Chimerism	**Conclusion**
Project: Chimer Marker Project_4-19-2011.SGC	**Panel:** SD_test_Chimer_PowerPlex_16_testSingleDonor	
User: Admin	**Quantification Type:** Height	
Report Time: 06/12/2012 - 12:58:25	**Coefficient of Variation:** 10.00%	**Comments**
Chimerism Type: %Donor Chimerism	**Locus Error:** 10.00%	
Confidence Level (MOE): 95%	**Measurement Error:** 5.00%	Date / Initial
Sample Name:	**Machine:**	**Authorization 1**
Run Time: - 00:00:00 -> - 00:00:00	**Patient Name:** John Doe	**Authorization 2**
Patient Age: 8	**Patient Sex:** Male	
Date of Transplantation: 1/1/2004	**Transplantation Type:** Peripheral Blood Transplant	

Sample Comments:

Donor: 1Donor.SG1

D_R_3_to_1.SG1

Recipient: Recipient.SG1

Marker Name	D3S1358 (13%)
%D CHM	73.62%
S.A. %D CHM	[71.78%]
LE	3.30%
S.A.LE	5.07%
ME	7.09%
Lim.Det.	3.20%
Ignored	No

|	%D CHM	S.A. %D CHM
Avg:	76.13%	75.61%
St.Dev:	5.15	5.27
CV:	6.76%	6.97%
MOE:	2.97 (95%)	3.04 (95%)
Info Loci:	14	14

The informative loci, or selected loci, of the post-transplant sample, and pre-transplant samples with the results for each locus on a separate page

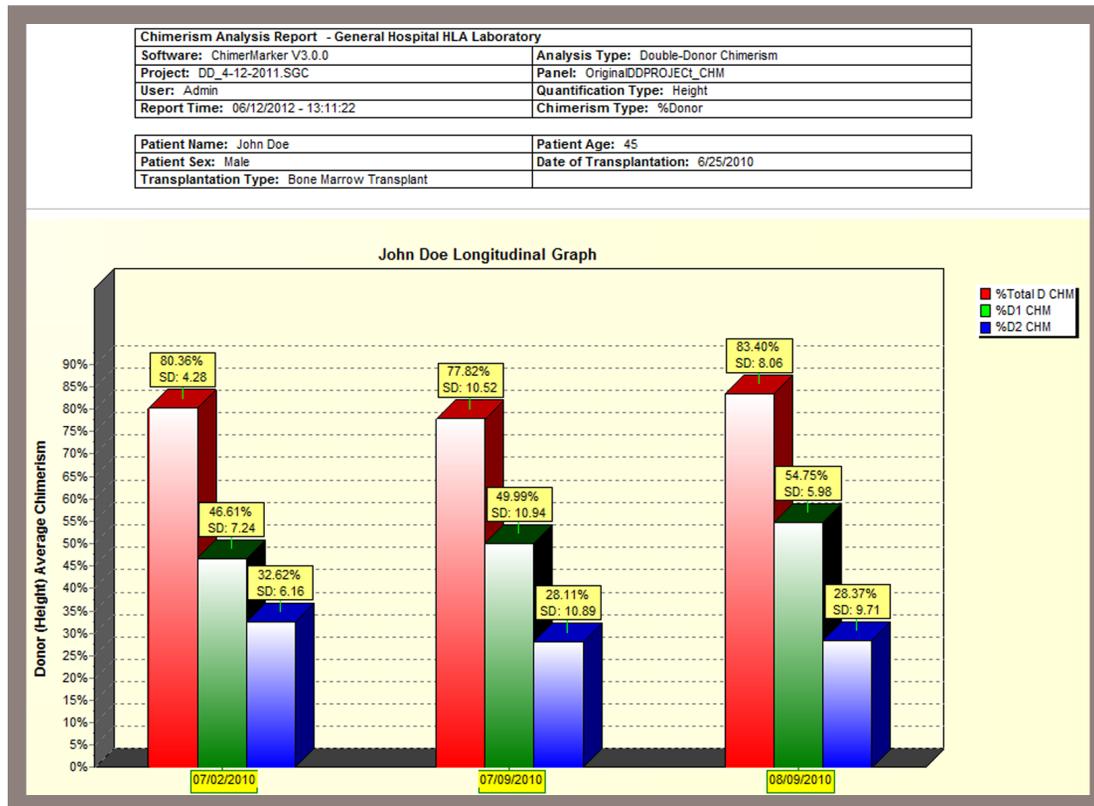## Monitoring Engraftment with Longitudinal Graphing

ChimerMarker software contains an integrated Longitudinal Report function. The software appends new sample files to an existing graph producing a time study monitoring % chimerism trend. Both the chimerism results and longitudinal graph are saved in a single project file for easy recordkeeping. Longitudinal reports are saved with the project and may be printed directly or saved as an image.

Single Donor Post-Transplant Monitoring Graph
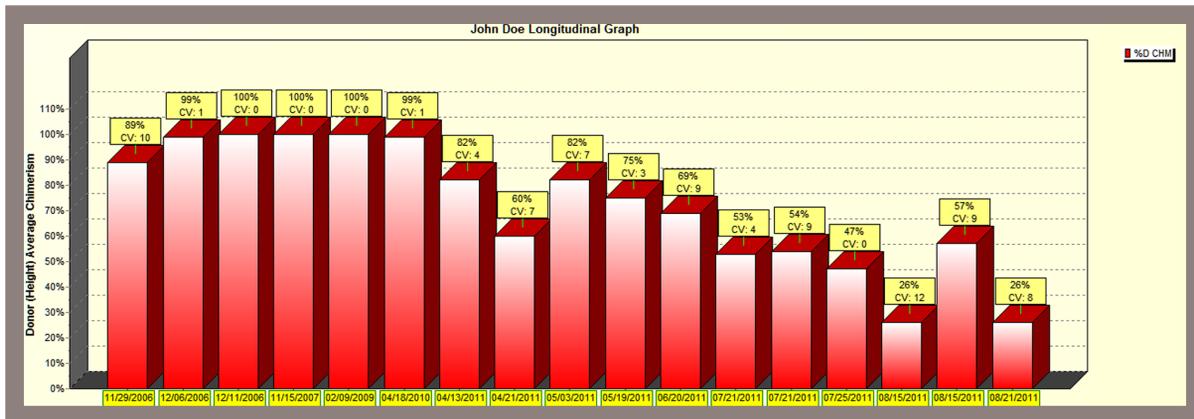
This example is a five year single donor study indicating an initial stability, but with a downward trend; alerting researchers to the change in chimeric status and potential need for intervention.

Double Donor Post-Transplant Monitoring Graph

Longitudinal graphing also simplifies engraftment monitoring for double donor cases. This example demonstrates a gradual increase in donor 1 and decrease in donor 2.

Audit Trail and User Management

User management provides control of user access rights and generates a user and audit trail for each project. Access rights for each user are determined by the administrator – providing assurance that unauthorized individuals do not accidentally alter a project or analysis parameters. User management also provides user ID and Organization name in the header of the final clinical research reports.

Password Protected Login

Login Record

Records Data Audit Trail Analysis
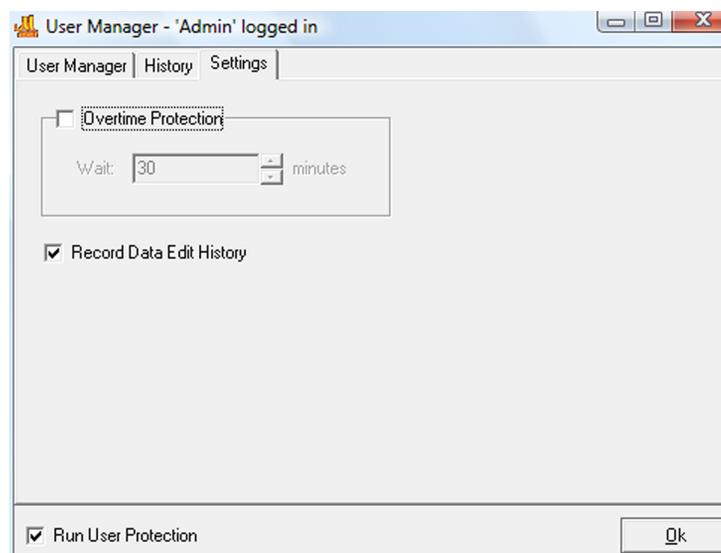
Preferences, Multi-Lineage Analysis and Automated Panel Construction

The selectable preferences include labeling peak ratios by height or area; with multi-lineage capabilities for chimerism analysis of T-cells, B-cells, and other cell type populations. These populations may indicate graft-versus-host disease (GVHD) or graft rejection. In addition, there are functions for comparison of samples at different time points to conduct longitudinal studies and produce a comprehensive graph. Case specific panels are automatically constructed, using Donor and Recipient allele calls.
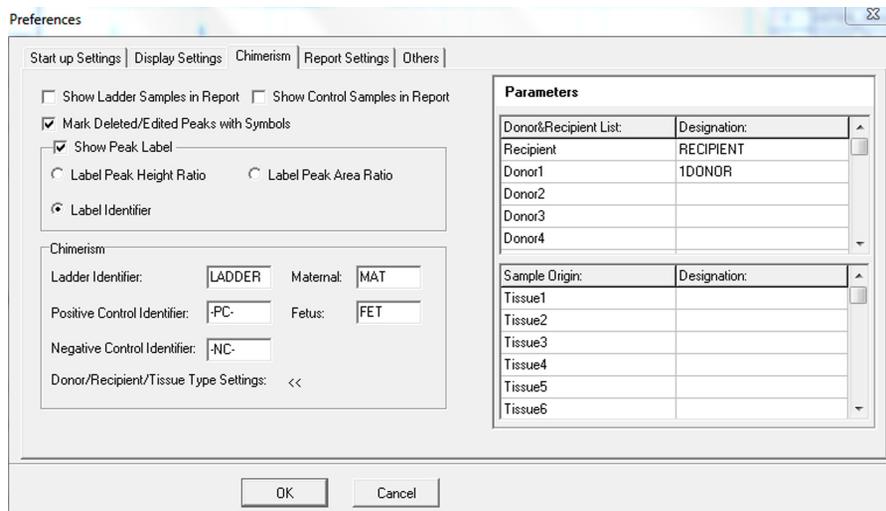
ChimerMarker recognizes up to five different tissue types with user-specified identifiers for multi-lineage analysis. Analysis and calculation time are reduced since samples from multiple cell types can be analyzed at one time when a Chimerism Panel is used.
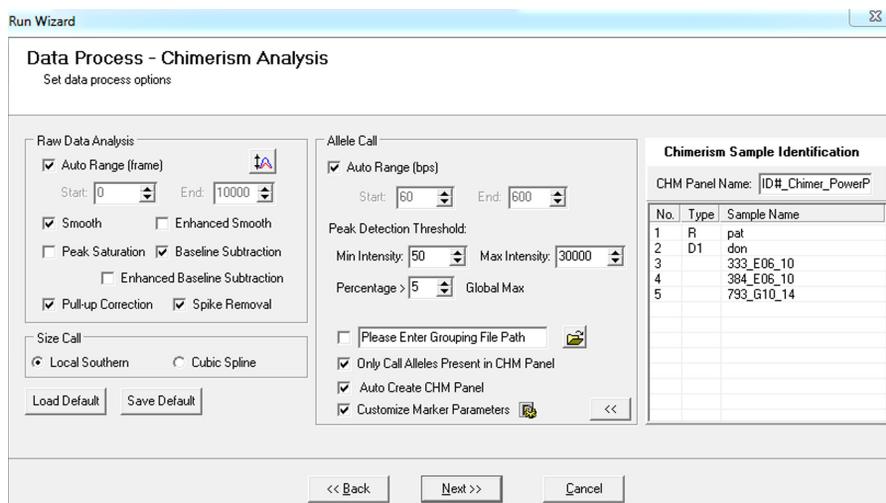
Analysis Parameters are easily selected and saved. The program corrects typical CE artifacts, automatically creates a panel from the allele calls of the pre-transplant samples and enables the user to keep the manufacturers recommended stutter filter values, or customize the parameters for each marker. Easily import pre-transplant genotypes from a formatted .txt file if the original CE files are no longer available.

Chimerism analysis parameters are selected from straight forward options to select % Chimerism and Quantification Type, Analysis Type (with or without deconvolution of shared peaks), Statistical Parameters, Error Thresholds, Limit of Detection cut off and subtraction of peak height attributable to stutter prior to chimerism calculation.

[†] Analysis of donor chimaerism levels following stem cell transplant: A comparison of ChimerMarker software and an in-house Excel based methodology. Kerry Wall, Joanne Mason, Susanna Akiki
West Midlands Regional Genetics Laboratory, Birmingham Women's NHS Foundation Trust, Edgbaston, Birmingham, B15 2TG, 2012 **Clinical Molecular Genetics Society/Association for Clinical Cytogenetics.**

[‡] Labs Report 85 Percent Reduction in STR Analysis Time with SoftGenetics' ChimerMarker Software, Udack Thomas, GenomeWeb March 23, 2012 **Franciscan St. Francis Health, HLA Laboratory** reported to BioInform

*D Kristt, J Stein, I Yaniv and T Klein. Assessing quantitative chimerism longitudinally: technical considerations, clinical applications and routine feasibility. **Bone Marrow Transplantation** (2007) 39:255–268.
D Kristt, et al. Quantitative monitoring of multi-donor chimerism: a systematic, validated framework for routine analysis. **Bone Marrow Transplantation** (2010) 45:137–147.
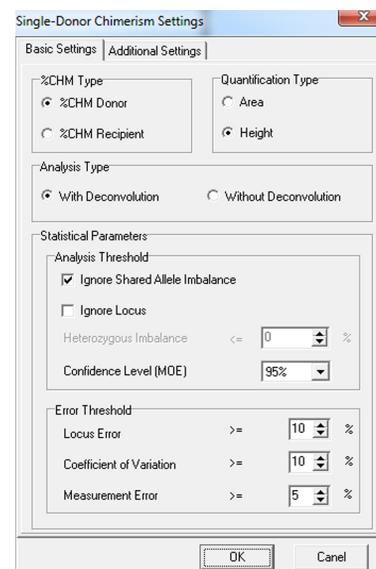
Maternal Cell Contamination

ChimerMarker provides accurate genotyping of maternal and fetal samples and uses rigorous mixture analysis algorithms to automatically detect and quantify MCC for cord blood banking or to screen samples prior to prenatal testing on chorionic villus and amniotic fluid samples. Sample grouping allows the user to analyze multiple cases in the same project. The MCC application is directly linked to the main analysis screen - removing the error-prone step of data transfer.
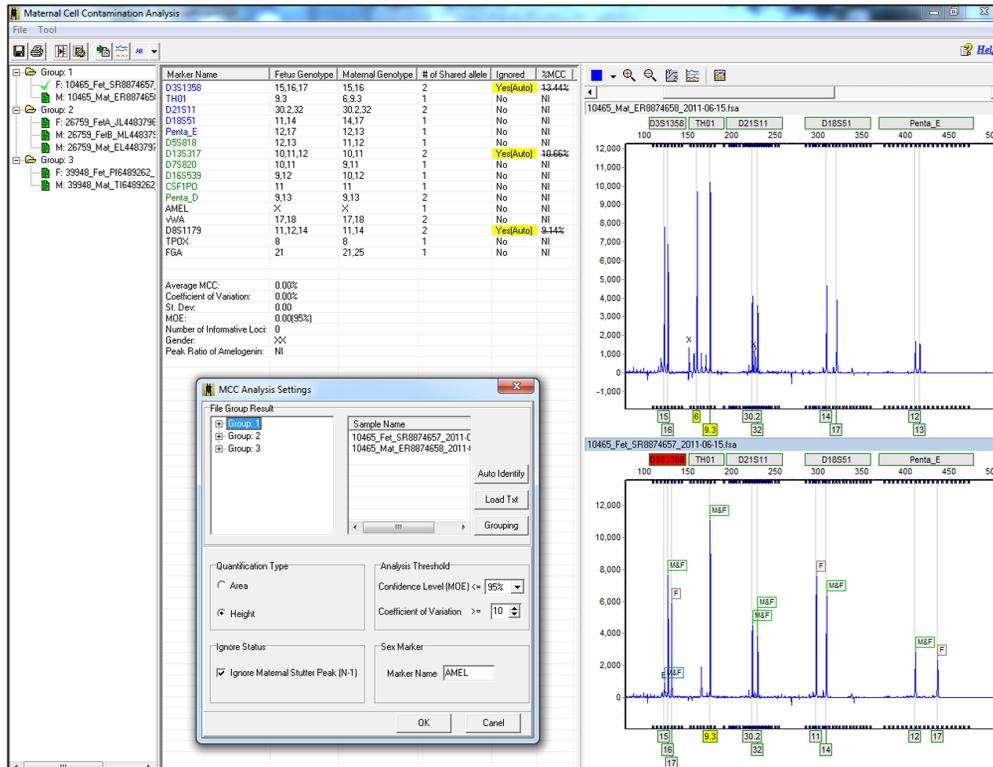
Computer System Requirements:

Windows® PC
OS: Windows® XP, Vista, Windows® 7

Processor: Pentium® III, 1 GHz
RAM: 512MB CPU
Available hard disk space: 20GB

Intel® Powered Macintosh®
OS: 10.4.6, with Parallels® desktop
for MAC or VMware Fusion®
or Apple™ Boot Camp
RAM: 2GB
Available hard disk space: 20GB

Storage Requirement Initial Analysis
Approximately 2500 KB for the initial project
Approximately 500 KB for each appended
  time sample
No Database Requirements

SOFTGENETICS®
Software PowerTools for Genetics Analysis

www.softgenetics.com

SOFTGENETICS®

Software PowerTools for Genetics Analysis