

ChimeRMarker™

Automated Chimerism Analysis Software



Quick Start Guide

ChimerMarker Work Flow

Load donor and recipient samples

Run wizard

Automated size and allele Call

Review data

- Rapid accurate allele and size calls
- Automated detection of informative alleles
- Linked chimerism calculations and statistics
- Audit trail and electronic record

Applications:

- Single Donor Analysis
- Double Donor Analysis
- Maternal Cell Contamination

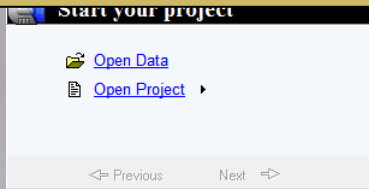
Select CHM Analysis Application

Clinical Research Report

Longitudinal Report

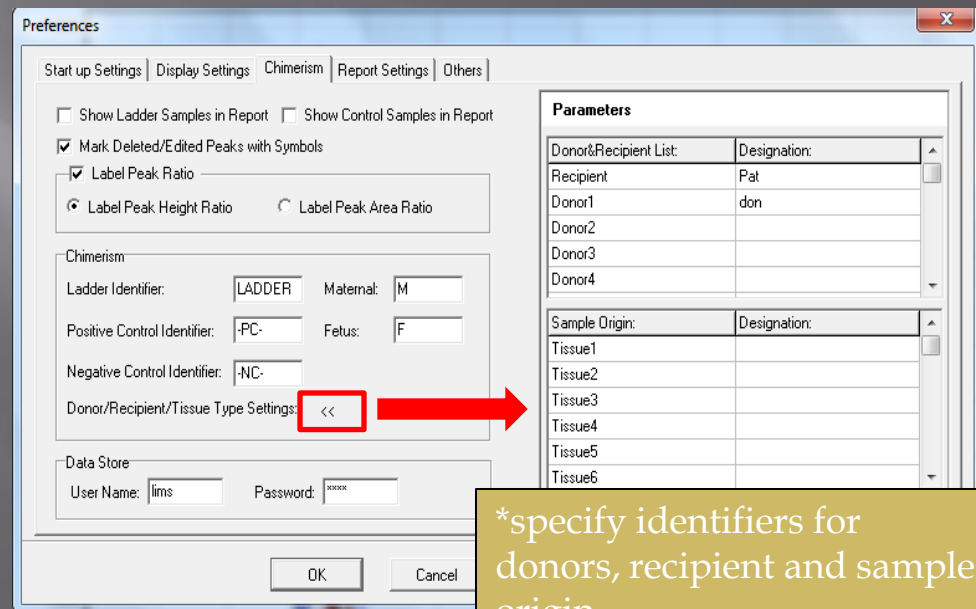
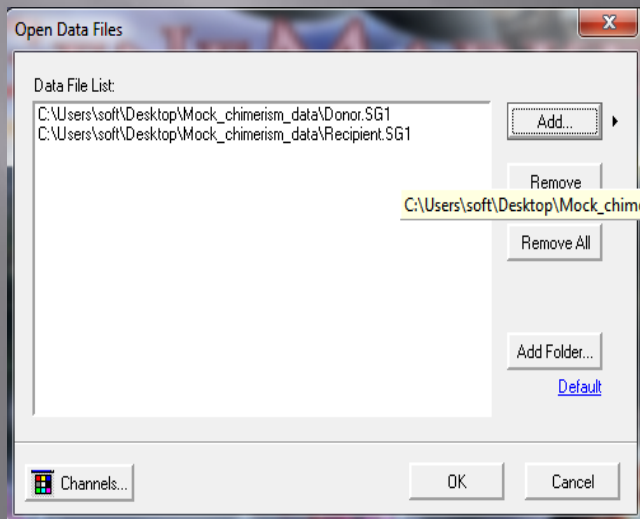
Loading Data and Specifying Identifiers

➤ Start Your Project:
➤ Open Data



View->Preference-> Chimerism

➤ Open Data Files:
➤ Add
➤ Select Files
(CTRL+A)



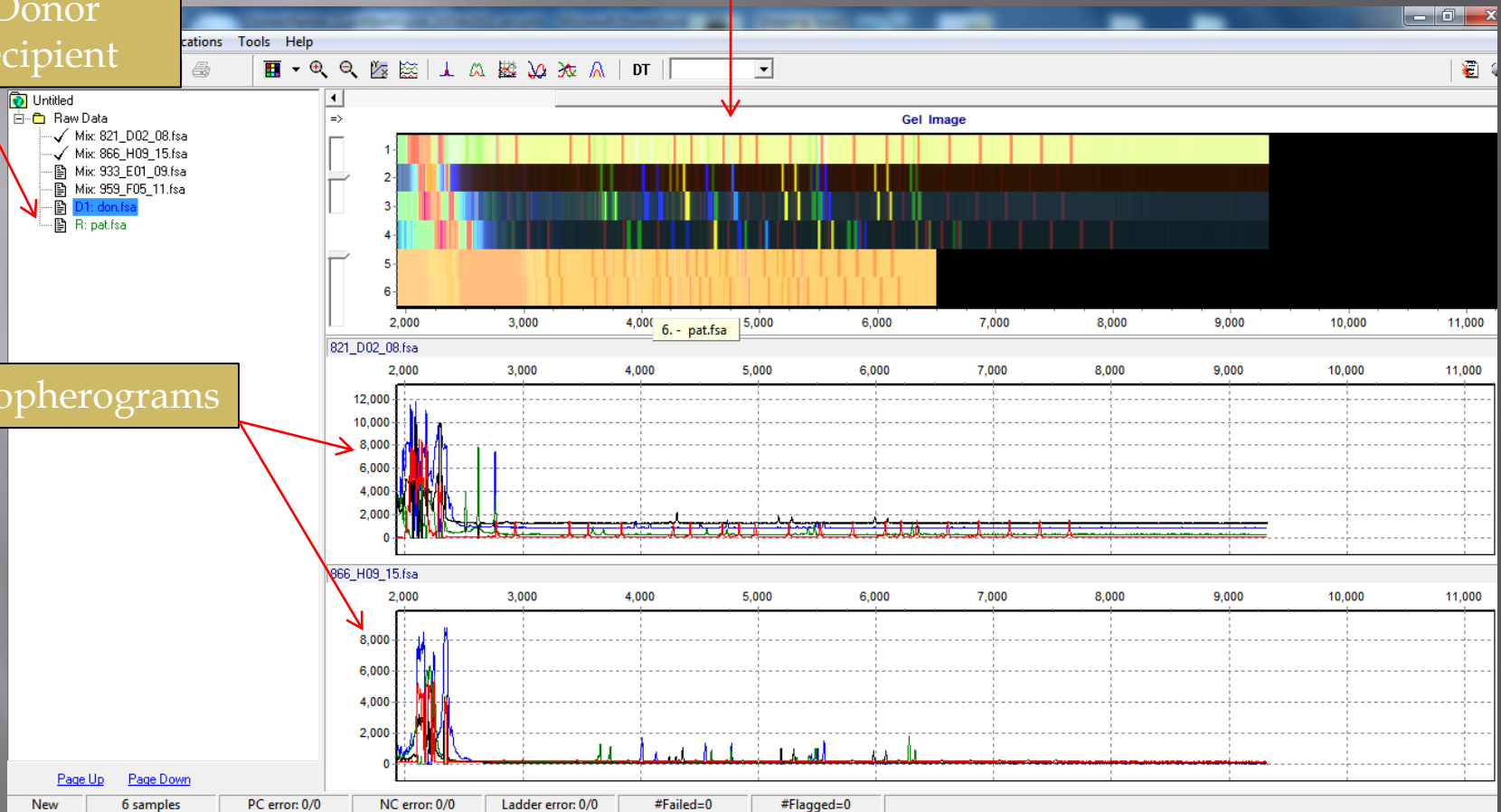
*specify identifiers for donors, recipient and sample origin

Raw Data Screen

Synthetic Gel Image

List of Files
D1= Donor
R=Recipient

Electropherograms



Size and Allele Calls using Run Wizard

➤ Click on Run Icon to begin



Run Wizard Template Selection:

- Select the appropriate Panel and Size Standard from Drop Down Menu.

IMPORTANT: Please calibrate panels to your instrument before proceeding. Refer to *Slide 7 or *Chapter 6 in User Manual or contact *Tech_Support@softgenetics.com

Run Wizard
Template Selection
Set the template of the project

Select an existing template or create one

- Identifiler
- Identifiler_MCC
- PowerPlex_16_ILS500
- PowerPlex_16_ILS600
- PowerPlex_ESI_17
- Profiler_HD400
- Profiler_Plus_HD400
- SGM_Plus

Use last template

Template Name: PowerPlex_16_ILS600
Panel: PowerPlex_16
Size Standard: ILS600
Standard Color: Red
Analysis Type: Chimerism

Save Delete

<< Back Next >> Cancel

Page 1

Page 2

Run Wizard
Data Process - Chimerism Analysis
Set data process options

Raw Data Analysis

- Auto Range (frame)
- Start: 0 End: 6000
- Smooth Enhanced Smooth
- Peak Saturation Baseline Subtraction Enhanced Baseline Subtraction
- Pull-up Correction Spike Removal

Allele Call

- Auto Range (bps)
- Start: 70 End: 400
- Peak Detection Threshold: Min Intensity: 15 Max Intensity: 6000
- Percentage: 10 Global Max
- Please Enter Grouping File Path
- Only Call Allele Present in CHM Panel
- Auto Create CHM Panel

Chimerism Sample Identification

CHM Panel Name: Chimer_DK_Profile1

No.	Type	Sample Name
1	R	pat
2	D1	don
3		037_G11_13
4		061_E08_10
5		384_E06_10
6		432_F11_11
7		558_E01_09
8		793_G10_14
9		823_D02_08
10		866_H09_15
11		933_E01_09

Load Default Save Default

<< Back Next >> Cancel

Data Processing:

- Select "Auto Create CHM Panel" to have ChimerMarker automatically create a Chimertyping panel and apply it.
- Deselect to manually create Chimertyping panel

Additional Settings:

- *Select *Auto Select Best Ladder* and *Auto Panel Adjustment*
- *These two options can only be used if *Panel is already calibrated*.

Additional Settings - HID Analysis
Set additional options related to the different analysis type

Allelic Ladder: NONE

Positive Control Template: NONE

Allele Evaluation

Peak Score: Reject < 1.00 Check 1.00 < Pass

- Auto Select Best Ladder
- Auto Panel Adjustment

<< Back OK Cancel

Page 3

See ChimerMarker Manual
Chapter 2 and 3

Review Size and Allele Calls

Main Toolbar

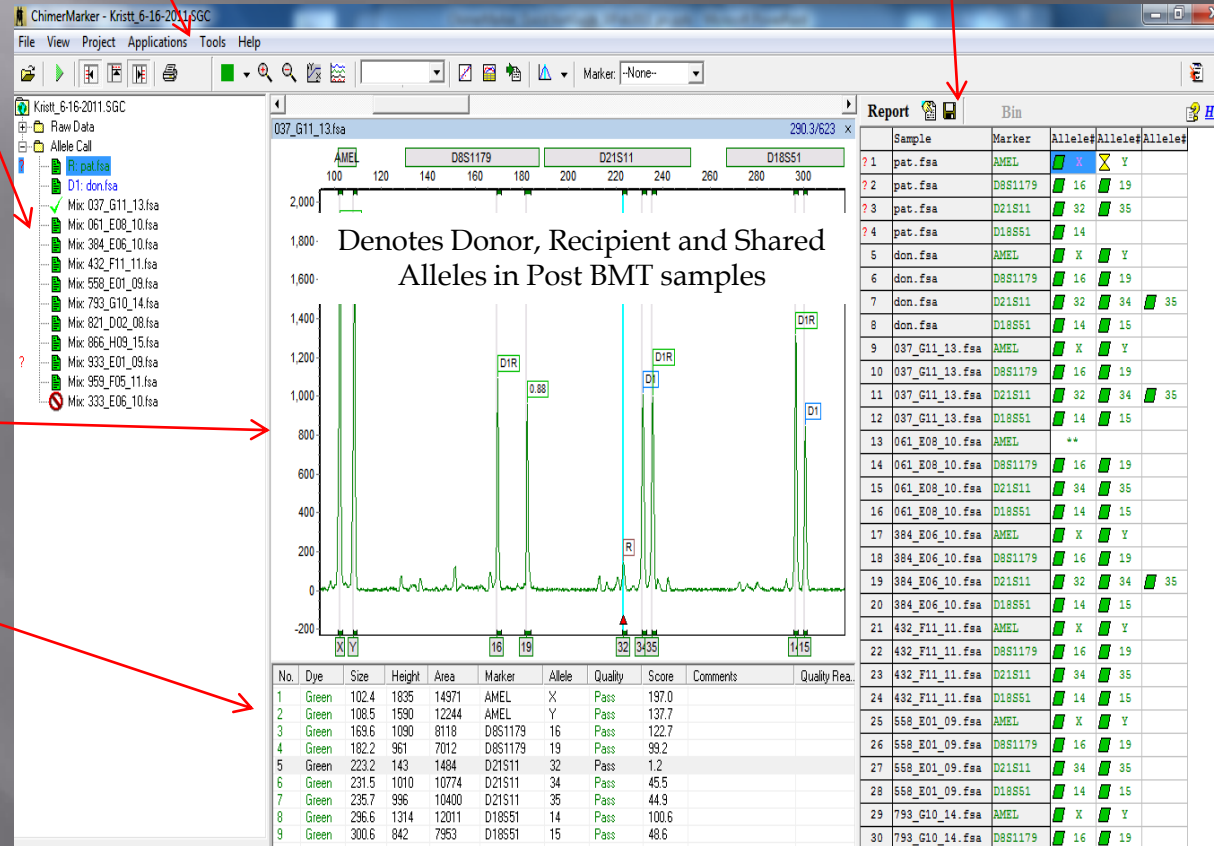
Allele Report Table

Size Called Samples

Green=High Lane Quality
Yellow=Requires Verification
Red=Size Did Not Occur

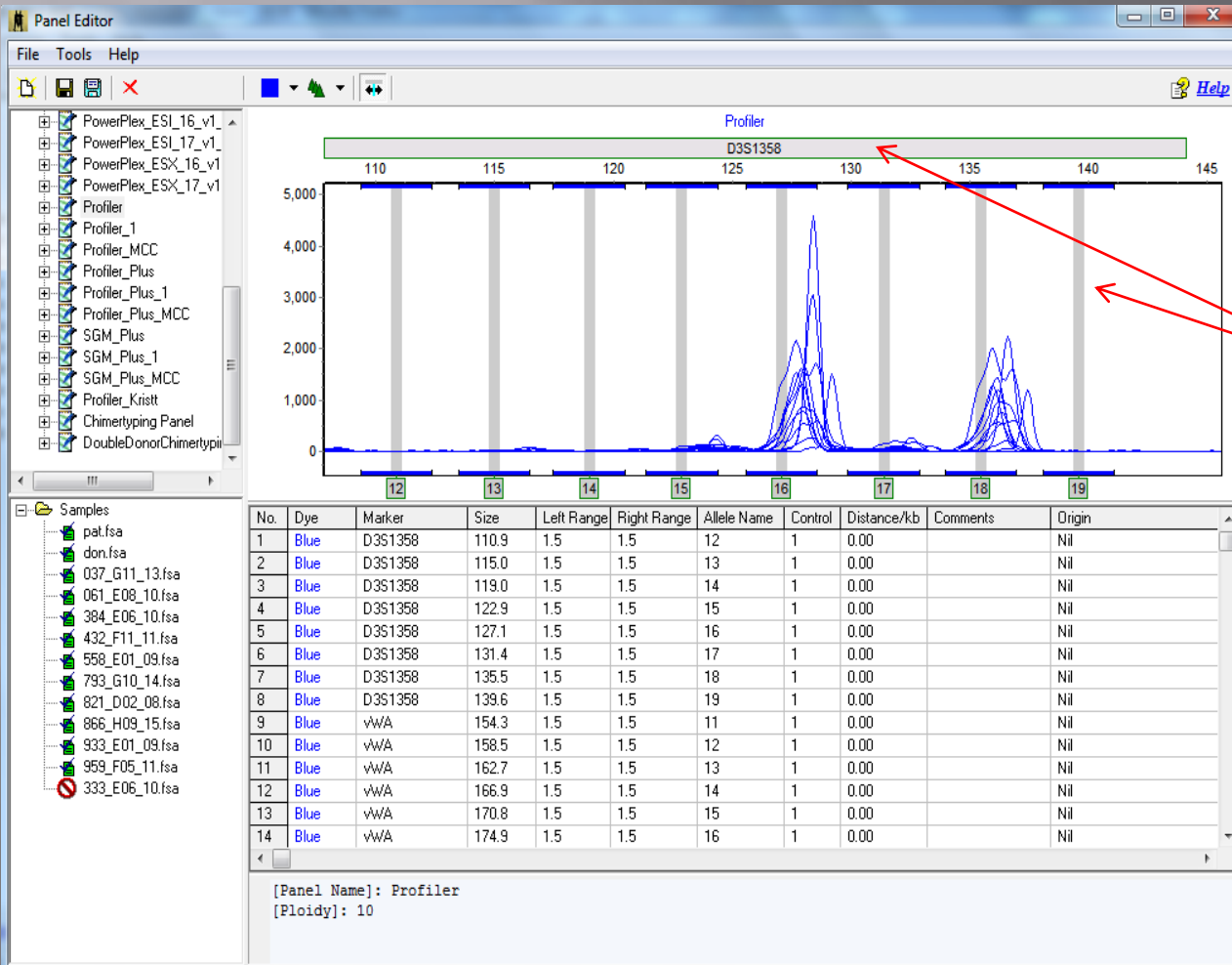
Electropherograms

Peak Chart/Table



Panel Editor

*Tools → Panel Editor → Select Panel of Interest under “Panel Template”



*If one or more allele is not aligned correctly to its bin:

- Adjust and calibrate panel
- Hold Shift key + Left-mouse click on Marker Label or Bin and drag it left or right to align it to the alleles.

- Adjust marker parameters → Filter out noise

Navigation

Zoom in:

-In electropherogram, hold left mouse click and drag a box from upper left to lower right

Zoom Out:

-In electropherogram, hold left mouse click and drag a box from lower right to upper left.

Scroll:

-In electropherogram, hold right mouse click and drag trace left or right.

One Color Viewing

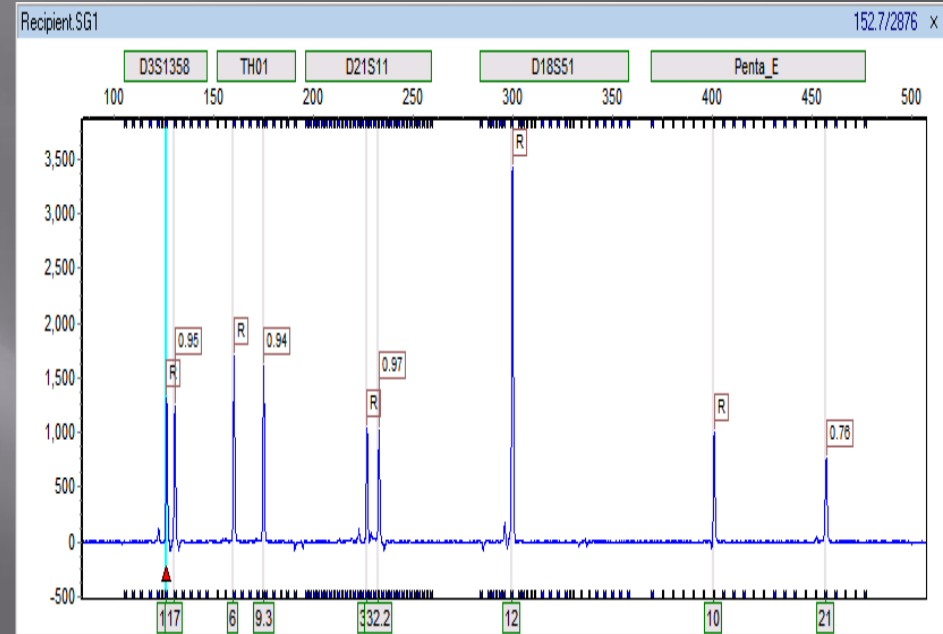
-Click the *Show Color* Icon



Hide Option:

-Click the *Show/Hide* Icons

remove flie list, gel image, report table



Making a Chimerism Panel

*If you selected "Auto Create CHM Panel" in the Run Wizard dialog, skip this step and move onto page 10

Tools → Panel Editor → File → *Create New Panel*

The screenshot shows the 'Panel Editor' window with a 'Create New Panel' dialog box open. The dialog box has the following fields and options:

- Name: Chimerism Panel
- Type: HID
- Method:
 - Manually Create
 - Automatically Create
 - Use All Samples
 - Use Selected Samples
 - Use Donor and Recipient Sample

The 'Parameters' section includes a 'Fixed Bin Width' of 0.5 and a table with the following data:

No.	Type	Sample Name
1	Donor1	Donor
2	Recipient	Recipient

At the bottom of the dialog, there are 'Ok', 'Cancel', and '<<' buttons. A red box highlights the 'Use Donor and Recipient Sample' option.

Specified Unique Panel Name

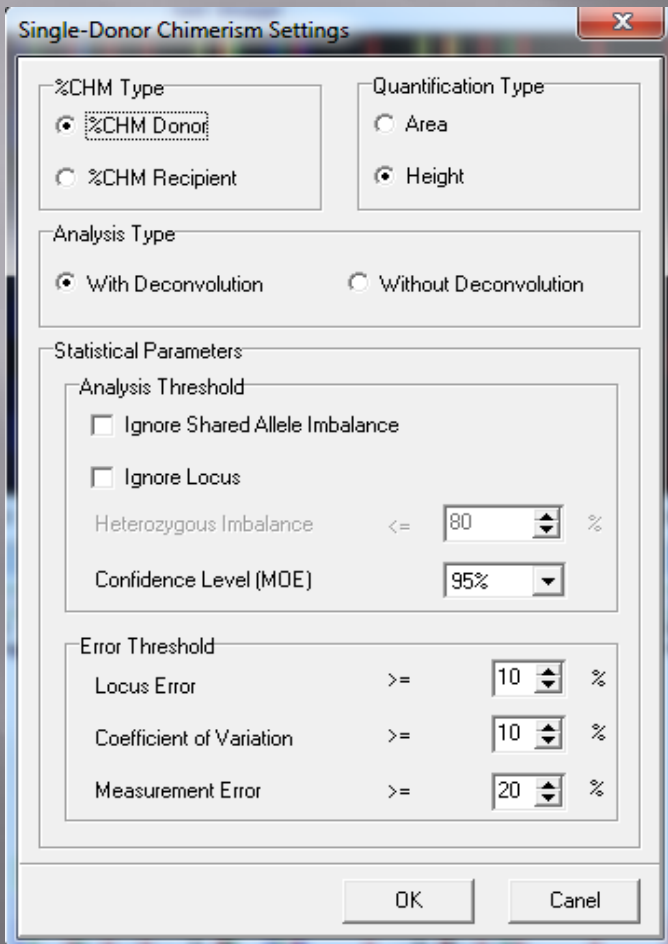
*Same procedure would apply to Double Donor Chimertyping Panel

Select Use Donor and Recipient Sample

Chimerism Analysis



Select from the Applications drop-down menu



Single Donor Chimerism Settings:

❖ %CHM Type: Calculate by % Recipient or % Donor (applicable to Double Donor)

❖ Ignore Share Allele Imbalance: Ignore markers that contain unique allele that is greater in intensity or area than shared allele.

❖ Quantification Type: Calculate Chimerism using Peak Area or Height (applicable to Double Donor)

❖ Analysis Type:

❖ *With Deconvolution*: Will use unshared allele information to deconvolute shared peak and calculate Chimerism

❖ *Without Deconvolution*: Will ignore locus with shared peak and only use unshared alleles

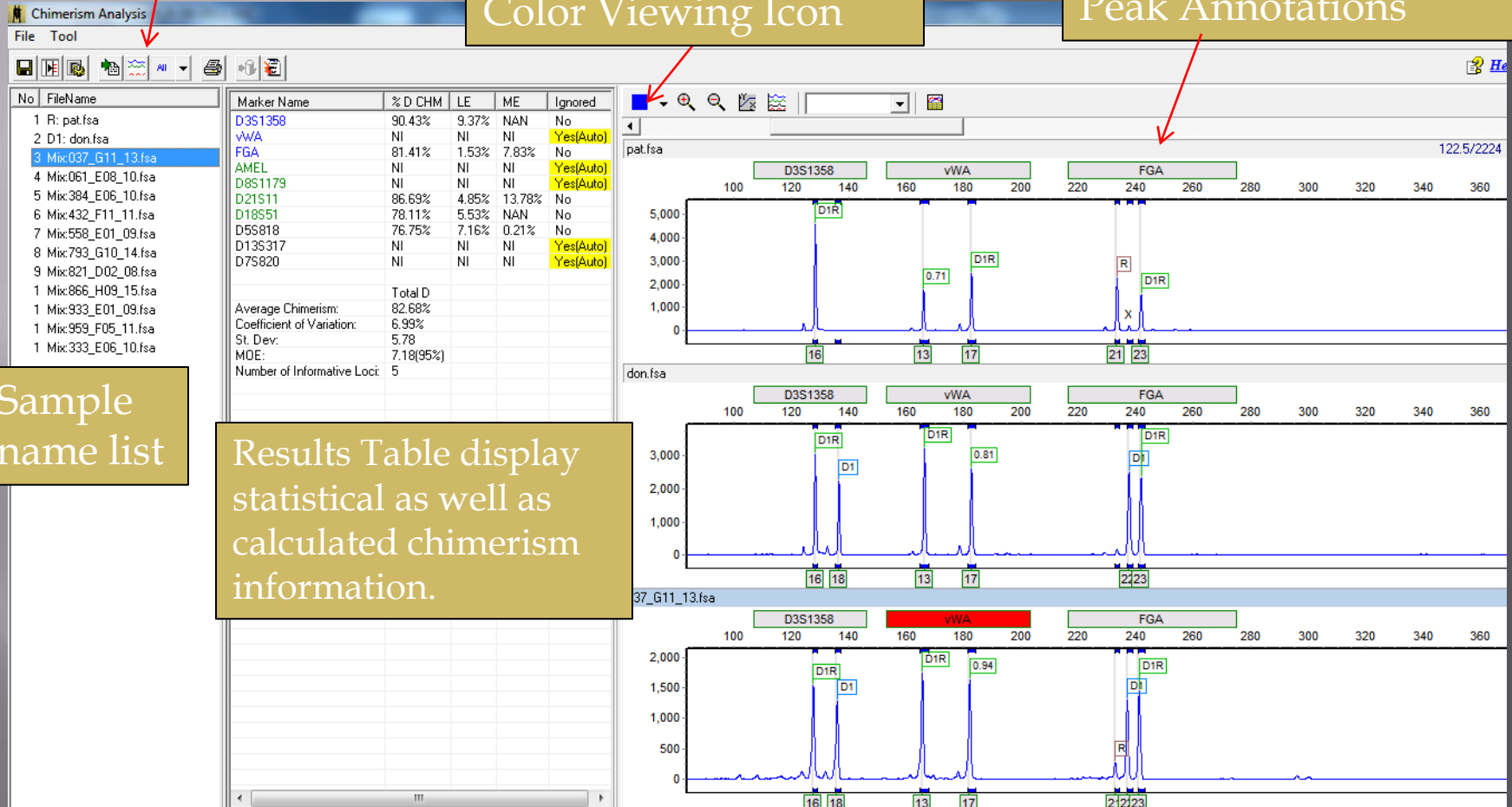
❖ Statistical Parameters: Parameters to flag locus or sample using set threshold. (applicable to Double Donor)

Single Donor Chimerism Analysis Results

Toolbar

Color Viewing Icon

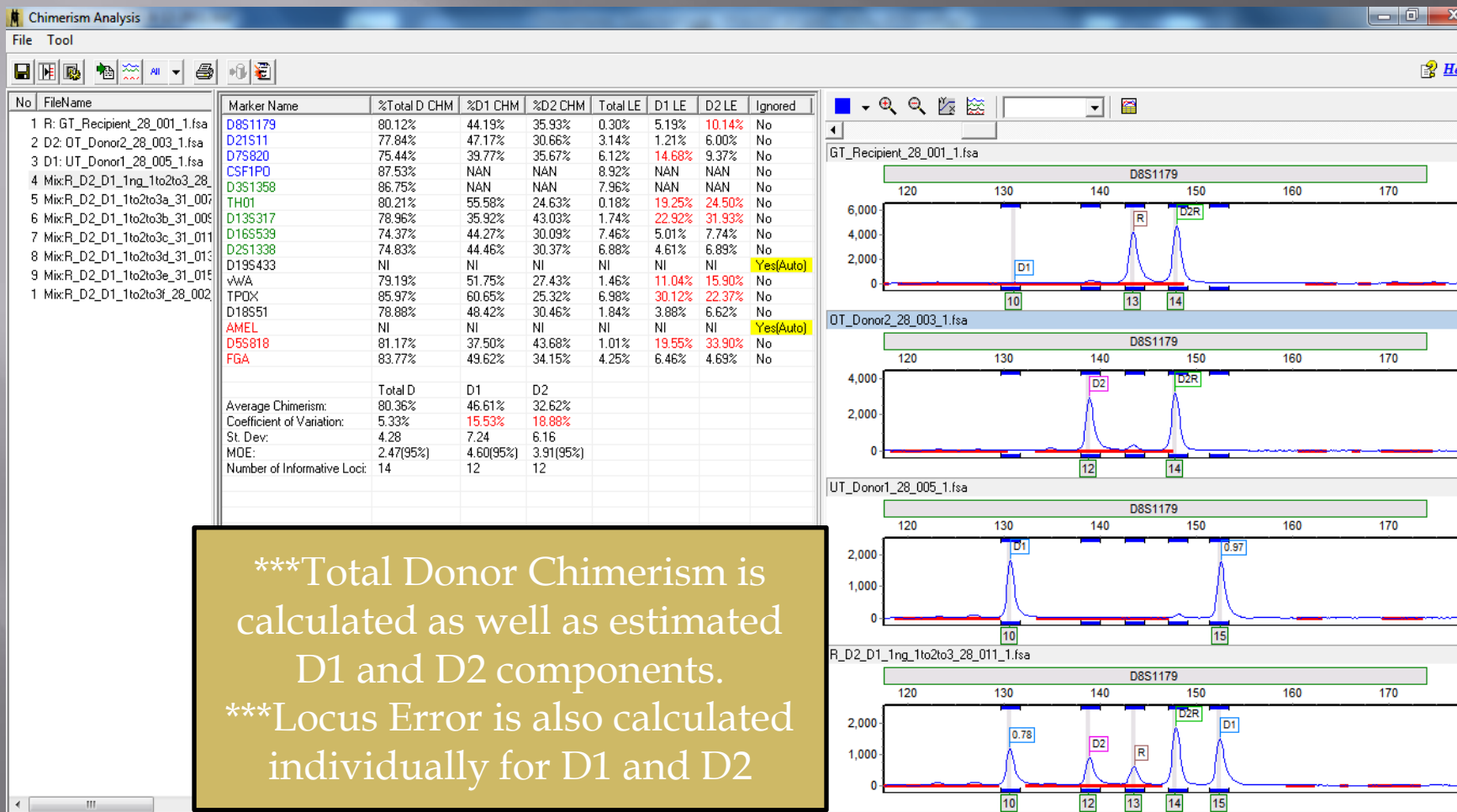
Electropherogram with Peak Annotations



Sample name list

Results Table display statistical as well as calculated chimerism information.

Double Donor Chimerism Analysis Results



***Total Donor Chimerism is calculated as well as estimated D1 and D2 components.
 ***Locus Error is also calculated individually for D1 and D2

Print or Save Report

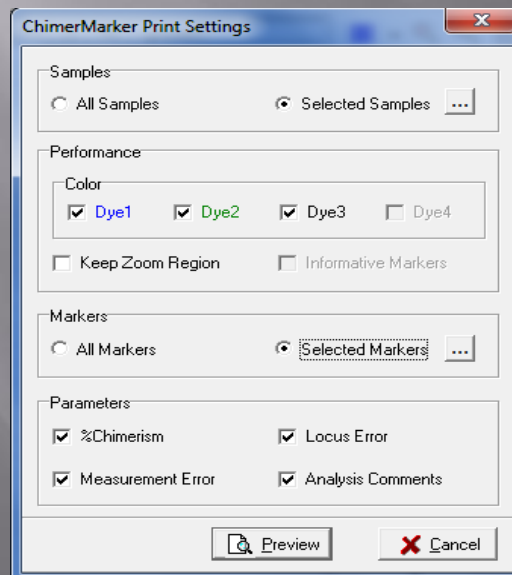
Save Report:
File→Save Report

Click on Print Icon



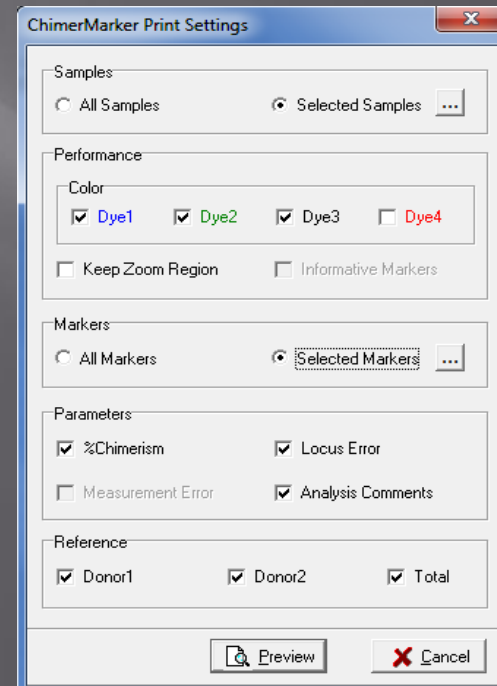
Print Options for Single Donor:

- Print all Samples or Select Specific Samples
- Print all Markers or Select Specific Markers
- Print all or only specific parameters.



Print Options for Double Donor:

- Print all Samples or Select Specific Samples
- Print all Markers or Select Specific Markers
- Print all or only specific parameters.
- Print all Reference components or specific components only.
- Keep Zoom Region prints current region of electropherogram



Comprehensive Report

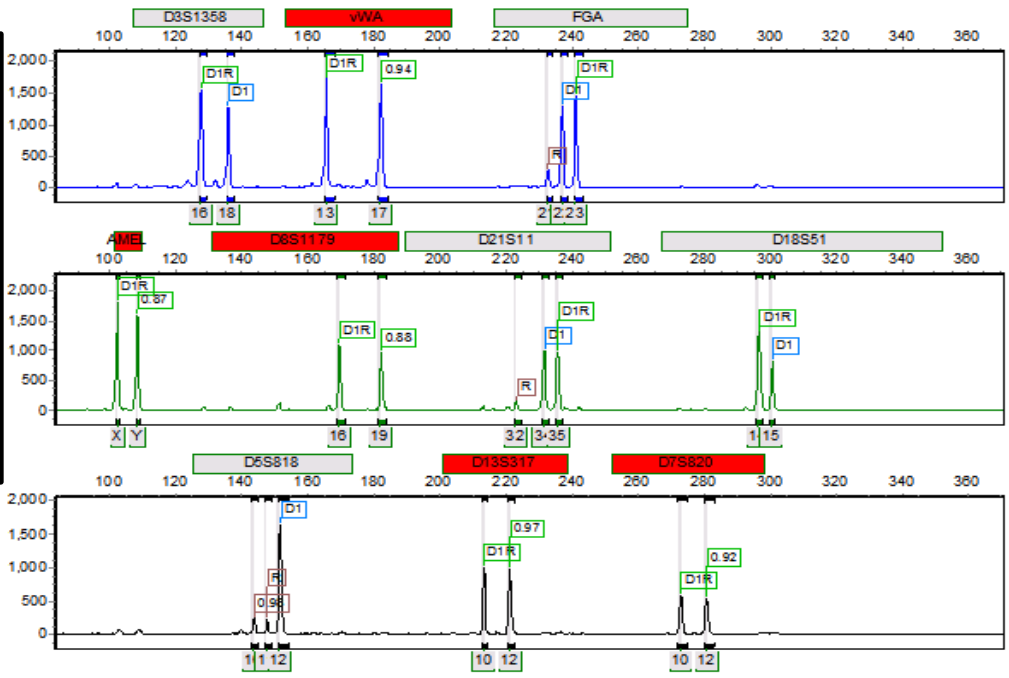
Header Information

Results Table

Chimerism Analysis Report - SoftGenetics	
Software: ChimerMarker V2.8.1	Analysis Type: Single-Donor Chimerism With Deconvolution
Project: Kristi_8-16-2011.SGC	Panel: DK_Test_CHM
User:	Quantification Type: Height
Report Time: 11/01/2011 - 10:10:00	Coefficient of Variation: 10.00%
Chimerism Type: %Donor Chimerism	Locus Error: 10.00%
Confidence Level (MOE): 95%	Measurement Error: 20.00%
Sample Name: 206037	Machine: demo_3100
Comments:	Run Time: 05/03/2011 - 11:52:15 -> 05/03/2011 - 12:33:49

Conclusion	
Comments	
Authorization 1	Date Initial
Authorization 2	

037_G11_13.sa



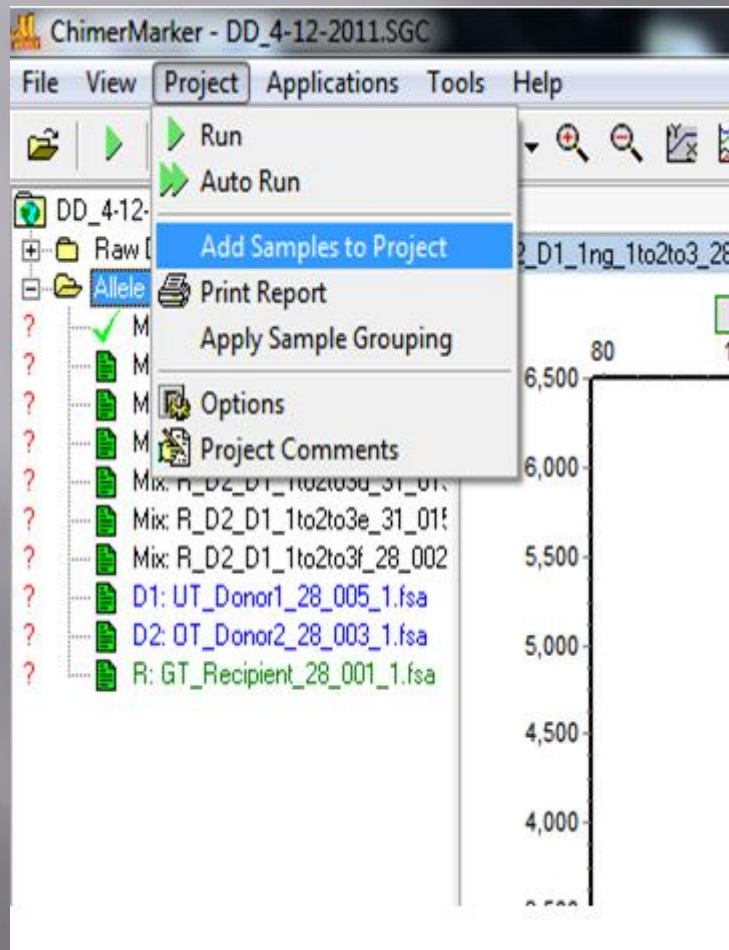
Marker	ME	LE	CHM	Ignored
D3S1358	NAN	9.37%	90.43%	No
vWA	NI	NI	NI	Yes(Auto)
FGA	7.83%	1.54%	81.41%	No
AMEL	NI	NI	NI	Yes(Auto)
D6S1179	NI	NI	NI	Yes(Auto)
D21S11	13.78%	4.85%	86.89%	No
D18S51	NAN	5.53%	78.11%	No
D5S818	0.21%	7.17%	76.75%	No
D13S317	NI	NI	NI	Yes(Auto)
D7S820	NI	NI	NI	Yes(Auto)

Average Chimerism: 82.88%
St. Dev.: 5.78
Coefficient of Variance: 8.99%
MOE: 7.18 (95%)
Number of Informative Loci: 5

Each dye color is printed separately with Peak annotation (D or R)

Average CHM, St. Dev., and CV

Long Term Monitoring: Adding Subsequent Samples



* No need to repeat analysis – Add Samples to Project appends the patient project with follow-up samples over time.

To add additional samples to a saved project:
Project → Add Samples to Project

Longitudinal Report

All samples within a project can be used to create a long term graph. For more information, please refer to The Longitudinal Webinar. For additional help/questions, please email tech_support@softgenetics.com

