

# Automated Chimerism Analysis Using ChimerMarker™ Software: A Concordance Study

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## Introduction

Chimerism is the presence of two or more genetically distinct cell types within a single individual. This condition is commonly produced in medicine by procedures such as blood transfusions and stem cell transplants. The general goal of these procedures is to supplement a deficiency in the recipient through the transfusion of cells or tissues from a healthy donor. Ideally, one tissue type will predominate and a stable equilibrium will be reached within the patient (i.e. 100% donor). However, there is always the risk of relapse or graft failure, requiring that chimeric patients be closely monitored after the procedure in case intervention is necessary<sup>1,2</sup>. Recently, PCR amplification of STR markers has replaced fluorescent *in situ* hybridization as the predominant method of quantifying chimerism.<sup>3,4</sup>

Unfortunately the analysis of qfPCR data for chimerism quantification currently has several practical limitations. Technicians must often implement several software packages – typically using a program to genotype samples, and then loading the results into a separate statistical analysis or spreadsheet program. Furthermore these programs often require manual determination of informative and uninformative markers, manual assignment of peak origins (i.e. donor or recipient), and unassisted mixture interpretation. Not only do these factors slow the analysis, but error-prone data transfer steps increase the risk of human error and may prevent consistent interpretation of results.

To resolve these problems, ChimerMarker software integrates raw data analysis, genotyping, and chimerism calculations into a single program. ChimerMarker automates the entire analysis procedure, from processing raw data through printing and sharing final reports. ChimerMarker automatically identifies informative markers and labels peak origins (donor, recipient, or shared). Chimerism calculations, including complex mixture deconvolution, are fully automated as well and are built directly into the program – preventing error-prone data transfers. Finally, ChimerMarker provides a wide suite of reporting options, suitable for both short and long-term studies. ChimerMarker is fully compatible with all major CE instruments and chemistries, and has demonstrated concordance with traditional quantification methods<sup>5</sup>.

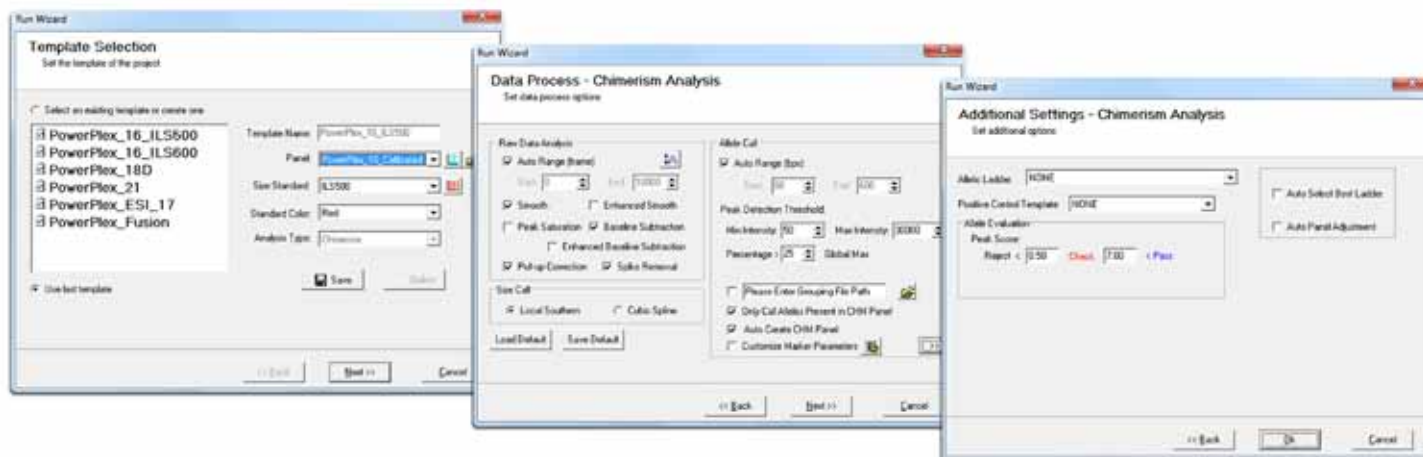
In this application note we demonstrate the workflow and accuracy of ChimerMarker by analyzing 11 .fsa validation files from DNA of known concentration amplified with the PowerPlex® 16 System (Promega, Madison WI). Data files and manual chimerism calculations from two technicians were generously supplied by Lia Mele at Azienda Ospedaliera S.S. Antonio e Biagio e C. Arrigo (Alessandria, Italy). The results, shown in Table 1, indicate that ChimerMarker was highly concordant with the known DNA concentrations.

Dilution	Known DNA (%)	Technician 1 (manual)	Technician 2 (manual)	ChimerMarker (% Chimerism ± StDev)
0	97.5	94.97	93.25	96.11 ± 6.05
1	95.5	90.93	92.06	91.12 ± 4.66
2	90.0	86.75	NA	86.51 ± 6.14
3	83.4	79.58	82.8	79.21 ± 6.01
4	66.7	62.81	NA	63.03 ± 7.05
5	50.0	48.13	43.85	49.92 ± 7.30
6	33.4	29.26	NA	32.98 ± 6.22
7	17.0	17.76	20.34	17.23 ± 5.70
8	10.0	12.3	NA	13.45 ± 5.27
9	5.0	5.2	8.22	4.87 ± 5.20
10	2.5	4.31	3.81	4.20 ± 3.84

**Table 1:** Validation Results. For each mixture the known concentrations of DNA are shown on the left. The concentrations of DNA as determined manually by two technicians and by a third technician using ChimerMarker are shown to the right.

## Procedure

1. Load raw data files (.fsa, .hid, .esd, .scf, .sg1) File  $\Rightarrow$  Open Data. Label Donor and Recipient files.
2. In the Run Wizard (Project  $\Rightarrow$  Run) select your pre-calibrated panel, size standard, and standard color, or select the corresponding pre-saved template.
3. Review genotyping results in the main analysis screen.
4. Select Applications  $\Rightarrow$  Single or Double Donor Chimerism Analysis.
5. Review results and preview final print report.

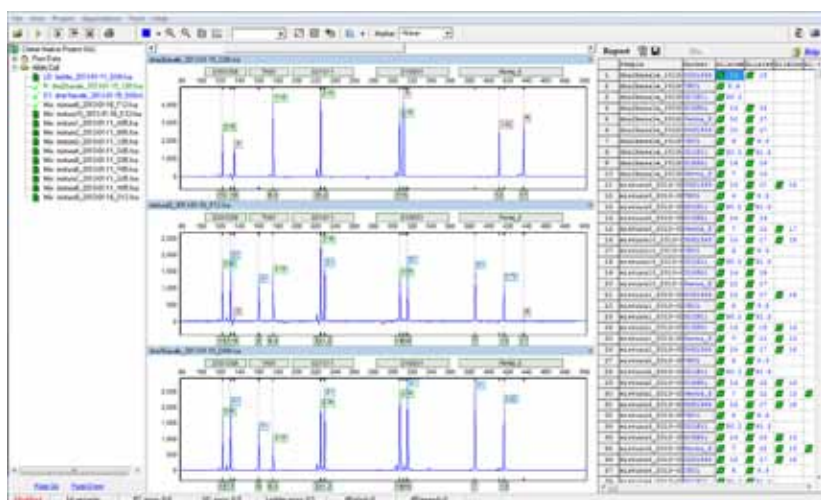


**Figure 1:** The three pages of the Run Wizard allow the user to easily select the panel and size standard corresponding to their chemistry, as well as the analysis parameters used for data processing. A case-specific chimertyping panel, with labeled bins for donor, recipient, and mixed peaks, is automatically created for each case.

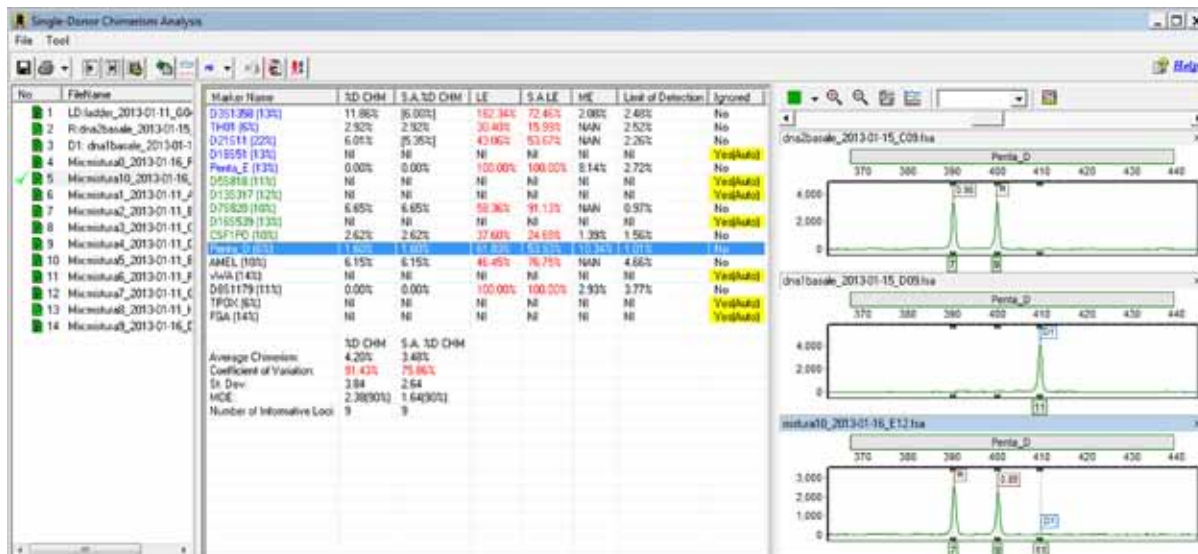
## Results & Discussion

Chimerism calculations were performed manually by two technicians, and by a third technician using ChimerMarker. The results in Table 1 demonstrate that ChimerMarker, on average, attained a high level of concordance to known DNA dilutions. Furthermore, the results show that two operators working by hand with the same samples often derived different results. ChimerMarker's automated workflow prevents the human inconsistencies that may lead to this variation, and the customizable analysis parameters can be saved to ensure that the analysis isn't left to interpretation.

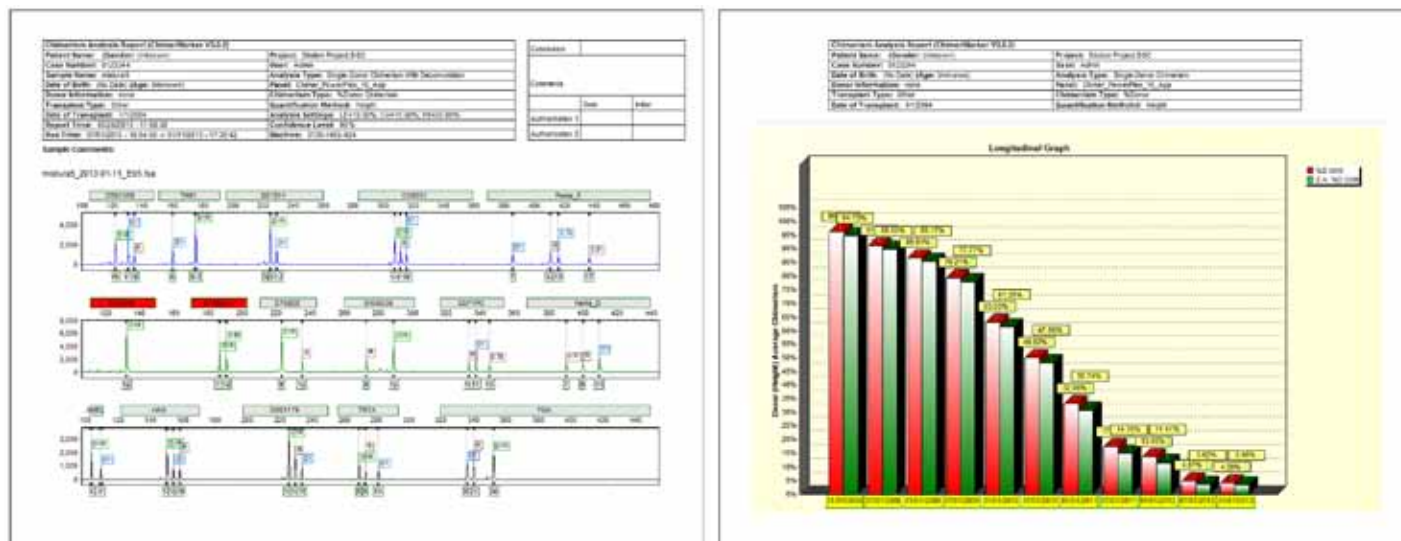
After data processing, the results can be initially reviewed in the main analysis screen (Figure 2). Peaks are automatically labeled according to their origin (donor or recipient), and may be flagged if customizable quality control standards aren't met. From the main analysis screen, the user can proceed directly to the Chimerism analysis application - no data transfer is necessary. Marker-specific and average chimerism values are automatically calculated and displayed for each sample (Figure 3). Results can then be saved in a variety of formats, including .txt and .xls, as well as customizable single time point and longitudinal reports (Figure 4). These features drastically reduce the time needed for analysis - by up to 85 percent<sup>6</sup> - and make ChimerMarker an efficient and accurate alternative for chimerism quantification.



**Figure 2:** Main Analysis window after data processing. Samples corresponding to the "recipient" (top), "donor" (bottom), and a mixture sample (middle) are displayed in the electropherogram. Notice that each peak has been automatically labeled according to its origin: D for donor, R for recipient, and D1R for shared peaks.



**Figure 3:** Automated chimerism calculations for each sample are displayed in the Chimerism Analysis window. Click on a marker to display the corresponding trace at that locus. Non-informative markers are automatically ignored and flagged. Markers may also be manually ignored by the user.



**Figure 4:** ChimerMarker offers several reporting options, including the two examples shown above. To the left is a single time point report, and to the right is a longitudinal report, which includes several time points. Each report includes an informative header, with patient, project, and transplantation information.

## References

1. D Kristt, et al. Quantitative monitoring of multi-donor chimerism: a systematic, validated framework for routine analysis. *Bone Marrow Transplantation* (2010) 45:137–147.
2. 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.
3. SJ Scharf, AG Smith, JA Hansen, C McFarland and HA Erlich. Quantitative determination of bone marrow transplant engraftment using fluorescent polymerase chain reaction primers for human identity markers. *Blood* (1995) 85:1954–1963.
4. C. Thiede, et al. Rapid quantification of mixed chimerism using multiplex amplification of short tandem repeat markers and fluorescence detection. *Bone Marrow Transplantation* (1999) 23:1055–1060.

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## References (cont.)

5. 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.
6. 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.

## Acknowledgements

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