

ChimerMarker™ Software for Automated Analysis, Chimerism Detection, Quantification and Long-Term Monitoring from Short Tandem Repeat (STR) DNA in Post-Bone Marrow Transplant Samples

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Introduction

Thousands of people suffer from leukemia, aplastic anemia and immune deficiency diseases in which the stem cells of bone marrow malfunction, causing defective blood cells that interfere with normal blood production and may invade other tissues. Treatments to destroy abnormal stem cells followed by transplantation of stem cells from compatible, healthy donor tissue have increased the success of treating these diseases. Chimeras, an individual composed of two genetically distinct types of cells, result from the transplant. Quantification of chimerism is used to monitor engraftment of donor cells following stem cell transplantation and is essential for early diagnosis of graft failure or relapse of the disease (1,2).

Chimerism analysis using a PCR-based method of short tandem repeat (STR) markers has grown to be the most widely used tool for chimerism analysis of stem cell engraftment (3,4,7), over fluorescent in situ hybridization (FISH) and other cytological methods. Not only are the available chemistry kits for genotyping relatively inexpensive, STR markers are highly polymorphic and increase the chance of finding informative loci for quantitative purposes. By using these informative markers, rapid detection and quantification of chimerism level in stem cell engraftment monitoring can be assessed with high confidence. However, there are multiple challenges to this analysis method. Some of these problems include selection of informative loci to measure chimerism, filtering stutter peaks, accurate genotyping of alleles, heterozygous imbalance, PCR amplification bias, and the repetition of quantifying chimerism for individual time points from multiple patients and multiple cell types. Combining robust data analysis, automated routine chimerism calculations (3,5,6,7), and flexible reporting would increase throughput and reduce errors.

Typically researchers will perform genotyping of samples in one software package and transfer allele call information to spread sheet or statistical software (2,4) to detect and quantify chimerism. These methods provide accurate analysis but require manual determination of donor, recipient and shared peaks; and transfer of allele call results (allele calls, peak heights, peak areas) is time consuming and may introduce errors. ChimerMarker™ software integrates the analysis workflow from raw data analysis to calculation of chimerism for single- or double-donor samples to final reporting. The program provides highly accurate size calibration and allele calling (genotyping), supporting multi-lineage cases (up to five different tissue types per study), linked to user-friendly chimerism analysis that automates detection of donor, recipient and shared peaks and applies repetitive calculations for chimerism detection. These results are summarized in a final report that contains a header documenting the user and analysis parameters, electropherograms and table with calculation results and summary statistics.

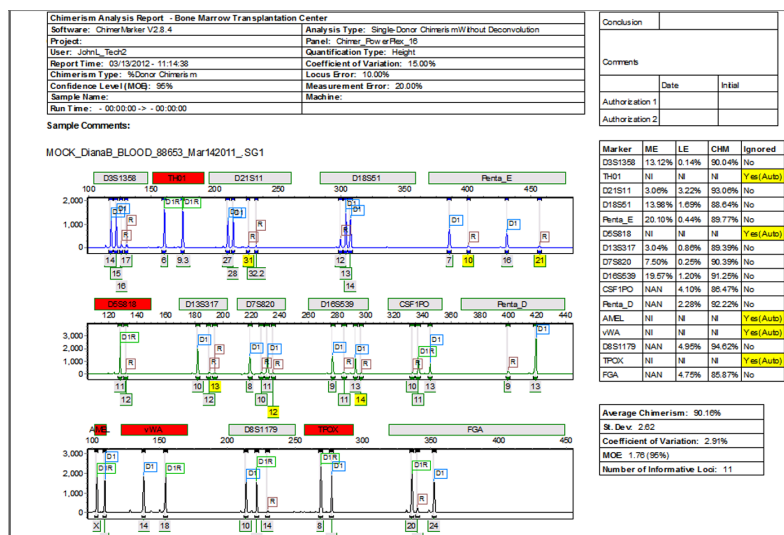


Figure 1: Comprehensive report for PostTX sample: includes header, electropherograms (traces) and report table. The header uses information from User Management and selected analysis parameters to record technician, project, time, date, and analysis settings for electronic record keeping. The electropherogram (trace) shows each dye separately with labels for D (donor), R (recipient), or combinations for shared alleles (DIR). The result table includes quality control metrics: coefficient of variation, standard deviation, average chimerism for sample, measurement error, and locus error for each marker and the total average Chimerism Percentage.

Procedure

1. Select Open Data to load raw data files (.fsa, .esd, .rsd, .scf, .sg1, .hid)
2. Select a Template (combination of panel and size standard) from the Run Wizard or proceed with customized settings (panels for analysis of commercially available human identity PCR kits are preloaded in ChimerMarker – or use the panel editor to construct custom chemistry panels)
3. Select “Auto Create CHM Panel”- specify Donor/Recipient samples and panel name
4. Select “Only Call Alleles Present in CHM Panel”
5. Review chimertyping results
6. Select Applications: single-donor or double-donor analysis to review chimerism quantification
7. Preview the final summary report

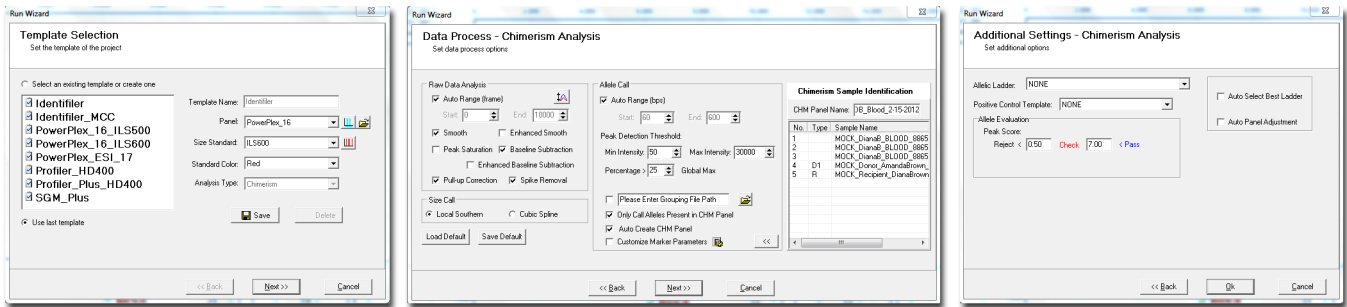


Figure 2: Three page run wizard provides user-friendly interface to set analysis parameters. Panels for commonly used human identity chemistries and size standards are preloaded in the software.

Results & Discussion

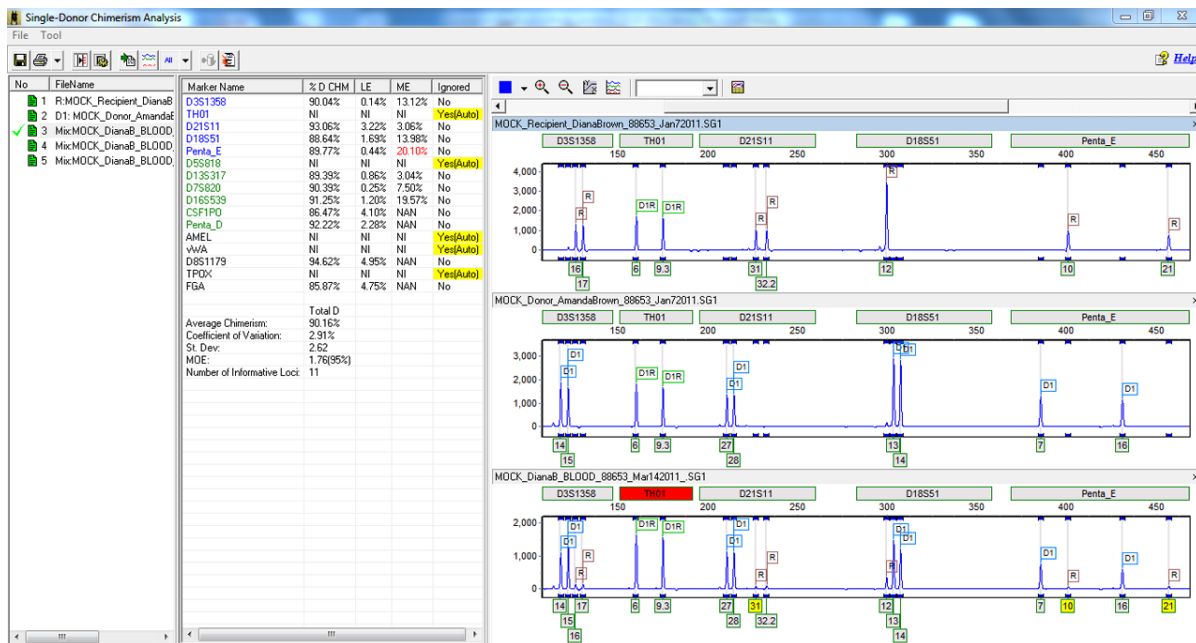


Figure 3: Post-transplant sample analyzed using a chimertyping panel: ChimerMarker automatically determines informative loci, differentiates and labels peaks for Donor1, Donor2 (only applicable to Double Donor Chimerism Analysis), Recipient, and reports the origin of the peaks for shared alleles (D1D2R or D1R, etc.) for each locus. Chimerism calculations are displayed for each marker along with electropherograms. Any non-informative markers are ignored and highlighted yellow with “Yes (Auto)” on the results pane and red marker label in the electropherograms.

Longitudinal Monitoring Procedures

1. Select Patient Information and fill in appropriate fields
2. Select Add Samples to add samples to graph
3. Adjust Display Settings if desired

Patient Information

Information

Patient Name: Sex: Male Female

Age: Date of Transplantation:

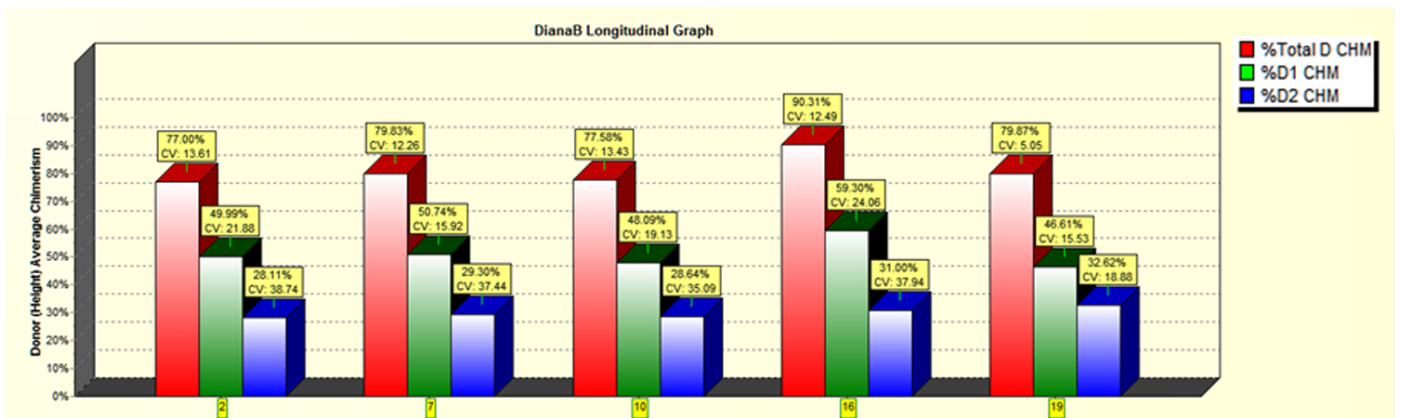
Transplantation Type:

Comments

Sample Group Selection

Sample Name	Date
<input checked="" type="checkbox"/> 97533_DB_1_Jan12_Y11	1/12/2011
<input checked="" type="checkbox"/> 97533_DB_2_Feb14_Y11	2/14/2011
<input checked="" type="checkbox"/> 97533_DB_3_Mar10_Y11	3/10/2011
<input checked="" type="checkbox"/> 97533_DB_4_Apr17_Y11	4/17/2011
<input checked="" type="checkbox"/> 97533_DB_5_May12_Y11	5/12/2011

Figure 4: Longitudinal monitoring takes two steps to set up: 1. Enter Patient Information (left) and 2. Add Sample(s) to generate a new graph or append a graph from an ongoing monitoring study.



No.	Sample Name	Week No.	Day No.	Date	Tissue Origin	CHM Total	CHM D1	CHM D2	SD Total	SD D1	SD D2	CV Total	CV D1	CV D2
1	97533_DB_1_Jan12_Y11	2	10	01/12/2011	Not Specified	77.00%	49.99%	28.11%	10.48	10.94	10.89	13.61	21.88	38.74
2	97533_DB_2_Feb14_Y11	7	43	02/14/2011	Not Specified	79.83%	50.74%	29.30%	9.79	8.08	10.97	12.26	15.92	37.44
3	97533_DB_3_Mar10_Y11	10	67	03/10/2011	Not Specified	77.58%	48.09%	28.64%	10.42	9.20	10.05	13.43	19.13	35.09
4	97533_DB_4_Apr17_Y11	16	105	04/17/2011	Not Specified	90.31%	59.30%	31.00%	11.28	14.27	11.76	12.49	24.06	37.94
5	97533_DB_5_May12_Y11	19	130	05/12/2011	Not Specified	79.87%	46.61%	32.62%	4.03	7.24	6.16	5.05	15.53	18.88

Figure 5: A longitudinal graph is generated using the samples in the project. The graph can display the number of weeks and days after transplantation, specific date format, and deconvolution of D1, D2, and Total Donor CHM percent (Top image). The summary table (bottom image) lists all results for the samples. Since the longitudinal tool is integrated in ChimerMarker, all information from raw data to final reports are kept in one complete project for easy record keeping.

Quickly Add Subsequent Monitoring Samples

1. Open saved project
2. Select Project in toolbar and “Add Samples to Project”
3. Import new sample(s)
4. Select Single Donor or Double Donor Chimerism Analysis
5. Open Longitudinal Report and select “Add Samples” to add new samples to ongoing graph

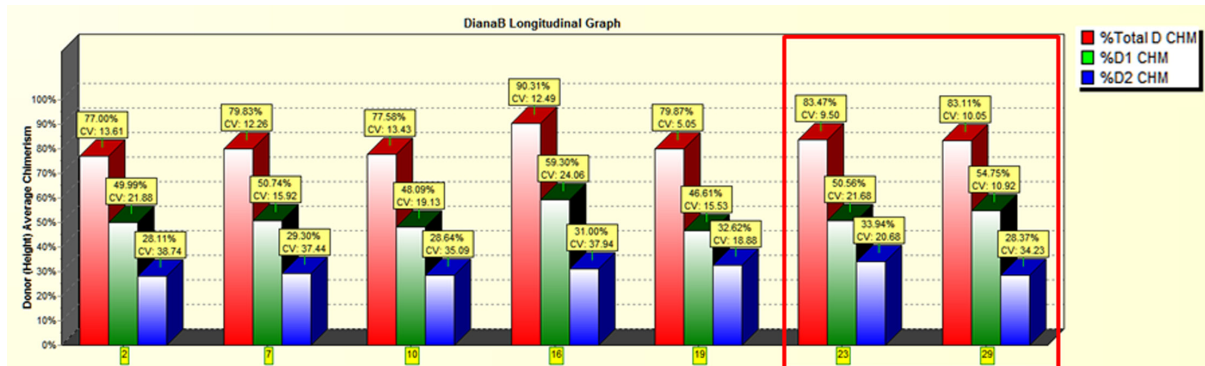


Figure 6: ChimerMarker can append additional samples to an existing project in seconds. This figure shows an appended longitudinal graph. Two additional samples for June and July were added to the graph.

ChimerMarker™, automated chimerism analysis software, integrates speed and accuracy with a biologist-friendly interface. The software can be used to monitor chimerism level in allogeneic stem cells transplant (SCT) or hematopoietic stem cells transplant (HSCT), bone marrow transplant (BMT, post bone marrow engraftment), and peripheral blood stem cells transplant (PBSCT) samples. The program automatically calculates percent chimerism and quality metrics, and has 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 chimerism analysis report. The chimerism analysis is completely linked to the main analysis screen, removing the error-prone step of data transfer from genotyping software to chimerism analysis software. ChimerMarker utilizes highly accurate size calibration and allele calling algorithms and labels donor and recipient peaks in the electropherogram. ChimerMarker is compatible with ABI®PRISM, Beckman-CEQ™, and MegaBACE® genetic analyzers, and custom primers or commercially available human identification chemistries for STR genotyping (including Identifiler®, Minifiler®, PowerPlex®16, PowerPlex®ESI).

References

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Acknowledgements

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