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Labs Report 85 Percent Reduction in STR Analysis Time with SoftGenetics' ChimerMaker Software

By Uduak Grace Thomas

Users of ChimerMarker, a short tandem repeat chimerism analysis software solution marketed by SoftGenetics, are reporting a significant reduction in the time required to analyze STRs in blood samples of patients who have undergone bone marrow transplants.

The tool automates the process of assessing the chimerism ratio — the proportion of donor cells relative to the host patient's own cells — in post-transplant cases based on the presence of STRs that are unique to both the patient and the donor, Don Kristt, head of molecular pathology at the Rabin Medical Center in Israel, explained to *BioInform* this week.

According to SoftGenetics, the software can be used to monitor chimerism levels in allogeneic and autologous stem cell transplants or hematopoietic stem cell transplants; bone marrow transplants; and cord and peripheral blood stem cell transplant samples.

SoftGenetics partnered with Kristt to develop the software, which it released last March (*BI* 3/18/2011). The company later added a module for testing fetal samples for maternal cell contamination prior to performing genetic testing for cystic fibrosis or other diseases (*BI* 9/2/2011).

The software provides capabilities for genotyping and chimerism analysis and tools to automatically identify donor and recipient peaks in samples following bone marrow transplants. It also calculates percent chimerism and quality metrics for single donor or double donor cases.

Dawn Wagenknecht, who supervises the HLA-Vascular Biology Laboratory at Franciscan St. Francis Health, told *BioInform* this week that her team was able to reduce the time required to calculate the ratio of donor to recipient cells in blood samples by as much as 85 percent.

She explained that the team ran parallel analyses of 10 blood samples using both ChimerMarker and a manual approach that the lab had used prior to purchasing the software, which involved manually sorting data generated by capillary sequencing in Excel spreadsheets, and then calculating the ratios either on the sheet or using a hand calculator.

In addition to the time savings, ChimerMarker also simplifies the analysis process because all the steps of the workflow are in a single package, she said.

The software also maintains records of the donor sample and the patient's blood before transplantation so that the results from subsequent tests after transplant can be compared to the initial samples, she said.

Prior to purchasing the software, Wagenknecht's lab had to use separate platforms to collect and analyze data.

Her team sequences its samples on a Life Technologies Applied Biosystems 3130 sequencer, and used Life Tech's GeneMapper tool to collect the data from the instrument and then transfer it to Excel for further analysis.

"We are looking at 14 different markers in the genome where we can find unique identities between the patient and the donors," in order to determine each cell's origin, she said.

That results in a lot of data per patient because each individual could have up to two alleles at each marker. Manually sorting through that data in Excel increases the likelihood of making a mistake in the analysis, she noted.

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Wagenknecht also said her lab had considered purchasing software from NicheVision, an Akron, Ohio-based company that also developing a tool for STR analysis, but those plans fell through because the software wasn't commercially available when the lab needed it.

The Importance of STRs

Analyzing the ratio of donor to recipient cells in patients who have had bone marrow transplants helps physicians ensure that transplanted cells are accepted by the host and don't result in complications such as graft rejection or graft-versus-host-disease — a potentially fatal condition where white blood cells in the donated bone marrow recognize their new host as "foreign" and begin to attack them.

The process begins by extracting DNA from bone marrow cells, amplifying the fragments using PCR, separating and detecting the STRs using electrophoresis, and then calculating the chimerism ratio for the donor cells, Kristt explained.

Repeatedly testing the patient's blood lets researchers follow the engraftment process — incorporation and proliferation of transplanted cells in a new host — over time and then compare later results to the sample prior to the transplant to determine the success of the procedure, he said.

Analyzing STRs — repeated patterns of two or more nucleotides — remains one of the "most effective" methods of monitoring donor/host ratios because it enables researches to quantitatively measure changes over time and compare one sample to another, he said.

According to an article published in *Bone Marrow Transplantation* in 2007 by Kristt and colleagues, the advantage of the STR-based approach to chimerism over others such as variable number tandem repeats is that it can "be utilized in virtually all donor:recipient combinations, regardless of gender, HLA or disease type."

The paper also explains that although VNTRs are similar to STRs, their larger repeat sequences "makes them more prone to inequalities during PCR amplification" which may in turn affect quantitation.

Furthermore, studies using methods such as real-time PCR to detect chimerism "have failed to show any clear superiority over STR-based analysis," the authors wrote.

Additionally, STR-based assays more frequently "detect mixed chimerism" — cases where there is a mix of donor and host DNA, "are more sensitive, and exhibit a higher incidence of informative markers" — genetic loci that are characterized by unique alleles that make it possible to distinguish between the donor and recipient, the researchers explain in the paper.