

Determine Concordance of Sample Replicates in GeneMarker® Software

April 2014

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Introduction

Clinical research labs and labs responsible for genotyping domestic or wild animal populations often run replicates of their CE samples. This technique lessens the impact of allele dropout and other common artifacts, and allows the analyst to confidently deduce a consensus genotype¹. Replicates are also commonly used to successfully analyze trace amounts of DNA, in some cases as little as 100pg^{2,3}. Despite these advances, comparing replicates manually can be both time consuming and error prone. For example, researchers must often use multiple software packages – one to genotype the data, and a second spreadsheet or statistical program in which to compare samples.

The Replicate Comparison Tool, now available in GeneMarker, automates replicate comparison and is built directly into the program. The analyst simply imports the raw data files, performs genotyping, and then automatically compares the replicates, all using a single program; no additional data transfer is necessary.



Figure 1: Overview of the Replicate Comparison Tool. Replicates are grouped together and compared in the electropherogram to the left. Allele calls for each replicate are compared to the right; differences are highlighted with yellow flagging.

Procedure

1. Import raw data files (.fsa, .hid, .esd, .scf, .sg1).
2. Perform genotyping by selecting the appropriate size standard, panel, and analysis parameters.
3. Review size and allele calls.
4. Group samples by navigating to Project -> Apply Sample Grouping.
5. Proceed to the Replicate Comparison Tool (Tools -> Replicate Comparison).
6. Select comparison parameters or use defaults (Figure 2).
7. Review final genotypes and address discordant markers.
8. Export Results.

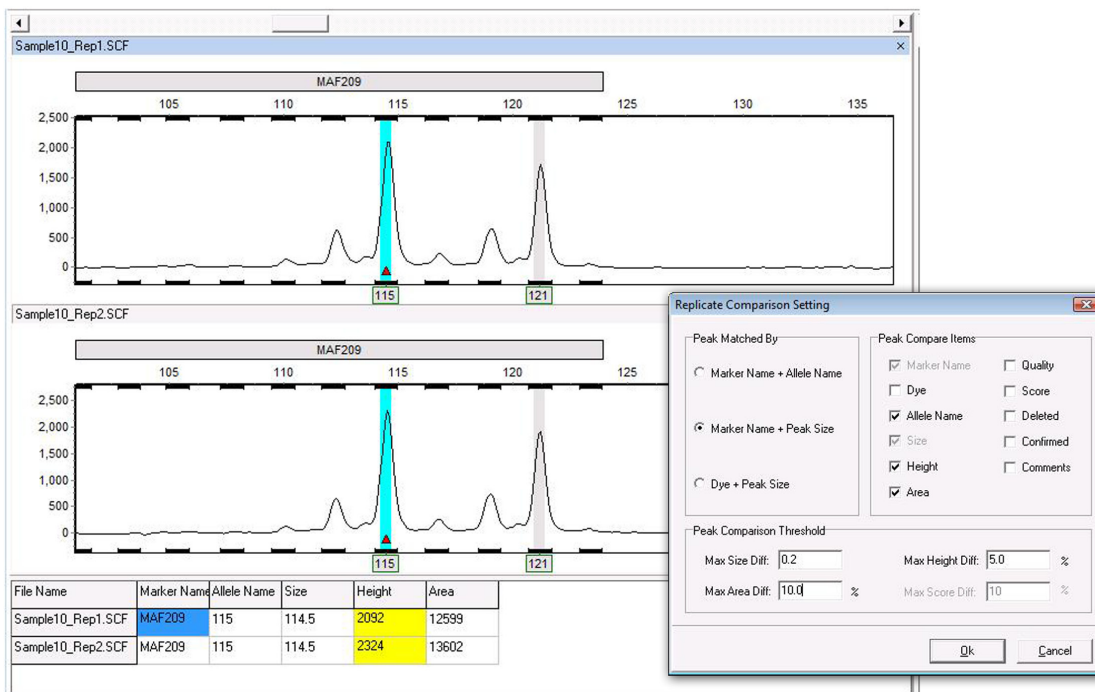


Figure 2: Use the Replicate Comparison Settings window to choose which parameters to compare for each replicate. For some parameters (size, area, height, and score) the user may set a range of allowable values.

The replicate comparison tool first enables the user to organize their samples into groups of two or more (Figure 3). The program then automatically compares the samples within each group based on the specifications selected in the settings window. The user may choose to compare only allele calls, or may also compare additional parameters, such as height, size, and area (Figure 2).

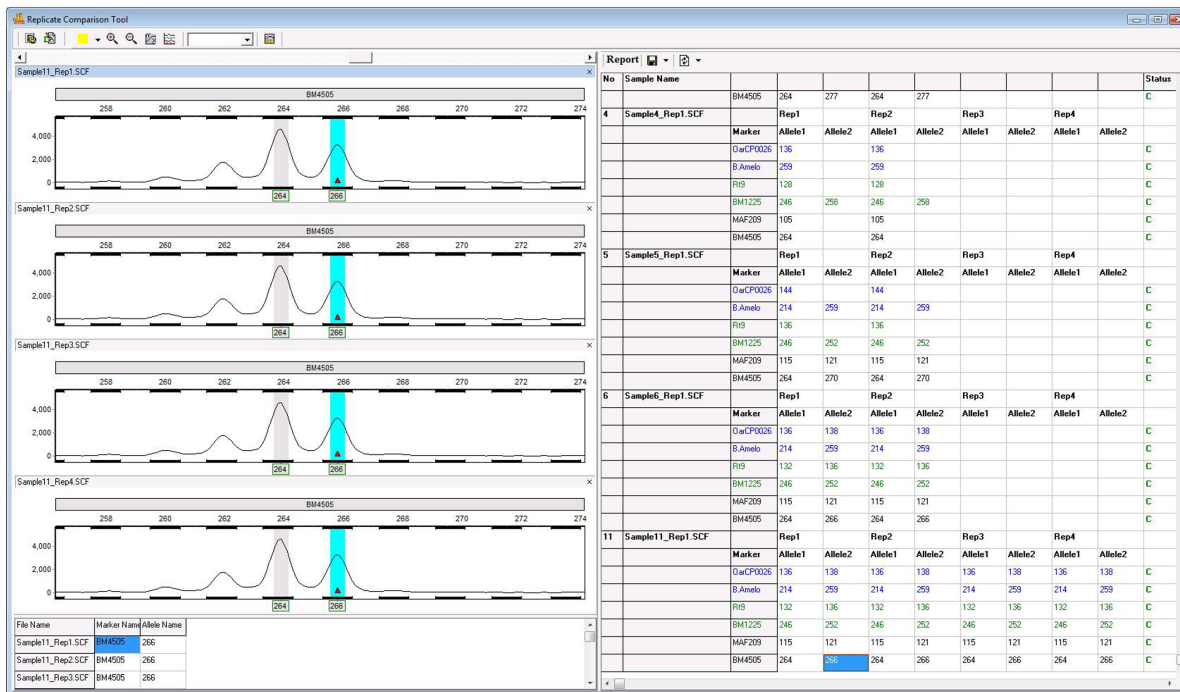


Figure 3: The replicate comparison tool can comfortably accommodate more than two replicates in each group. Shown above is a project in which up to four replicates were processed for each sample.

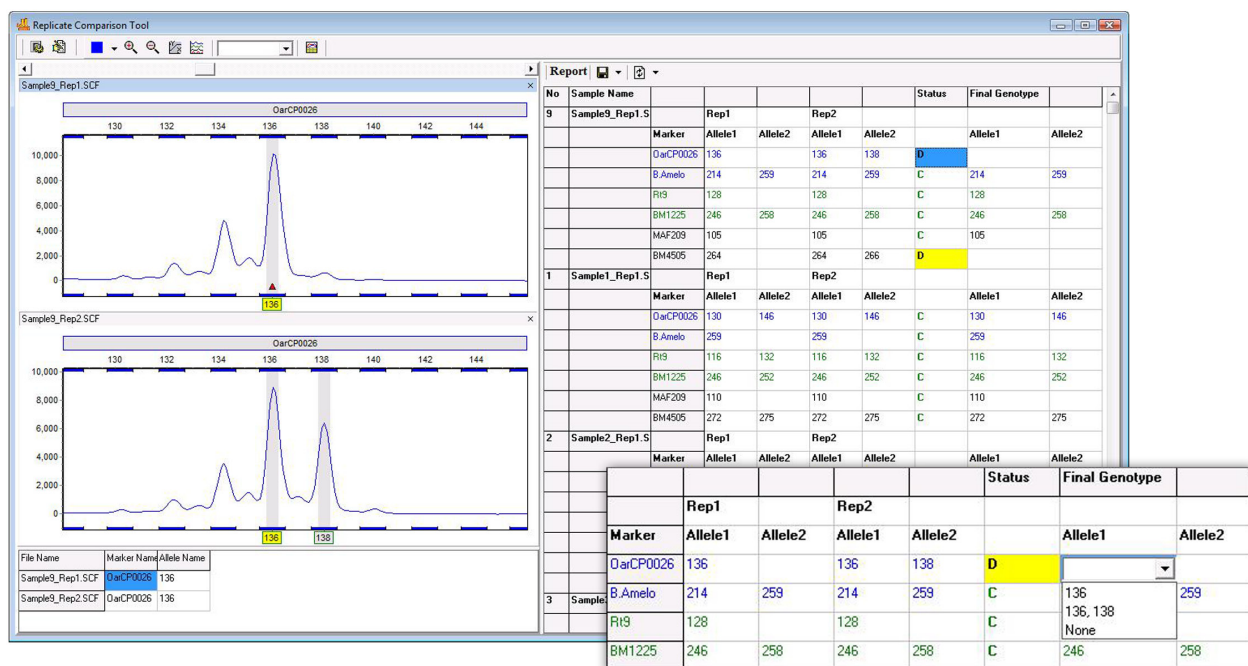


Figure 4: The report table. When the program detects a discrepancy between two replicates, the errant marker is flagged and the status column is filled with a D, for discordant. The user may manually choose the call for that locus using a dropdown menu.

Results & Discussion

Results are automatically displayed in the informative report table (Figure 4). The status column for a specified marker will display a C for Concordant if the replicates have the same call, a D for Discordant, if the call is different, and N, for Null, if only one replicate has allele calls (i.e. the other replicates failed). For C and N calls, the Final Genotype column is automatically filled with the consensus genotype. At discordant (D) markers, the analyst may use their expertise to select the most accurate call from a dropdown menu.

The report table is closely linked with the electropherogram, allowing the user to quickly and efficiently analyze markers of interest. GeneMarker is also preloaded with several clinical research and ecology/environmental research applications, including modules for FragileX, MLPA, Microsatellite, Trisomy and AFLP analysis. GeneMarker supports numerous reporting and saving options, including customizable print reports, excel exports, and text exports.

Acknowledgements

Special thanks to Tricia Frye at DNA Diagnostics Center (Middlefield, OH) for helpful discussions.

References

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