OSIRIS: A valuable tool for assessing donor cell engraftment after stem cell transplant

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OSIRIS is free, open source STR analysis and quality control software designed to increase the efficiency and accuracy of STR DNA analysis using Applied Biosystems 310, 31xx, 3500 and 3700 series Genetic Analyzers.

OSIRIS version 2.3, developed in collaboration STR analysis laboratories and clinical laboratories specifically addresses analysis of donor/recipient chimerism samples, low level DNA analysis, and editing efficiency. OSIRIS v. 2.3 increases technician efficiency and consistency, and improves sensitive low-threshold analysis using dynamic baselining in the identification of low level peaks and artifacts. OSIRIS has been validated for clinical testing of bone marrow engraftment and has been validated by laboratories as an expert system for single source DNA analysis.

With resources limited, and the volume of STR DNA profiling increasing, there is a need to increase DNA analysis efficiency and accuracy. OSIRIS, the Open Source Independent Review and Interpretation System, is freely available software for the analysis of STR profiles produced using Applied Biosystems 310, 31xx, 3500 and 3700 series Genetic Analyzers, and can be downloaded at http://www.ncbi.nlm.nih.gov/projects/SNP/osiris/. OSIRIS analyzes both .fsa and .hid format files produced by the collection software, using an independently derived mathematically-based sizing algorithm[1], including sophisticated dynamic base lining. OSIRIS supports numerous commercially available marker kits including kits favored by biomedical and identity testing laboratories. Supported kits include, among others, AB Identifiler™, AB GlobalFiler™, Promega PowerPlex16™, and Promega Fusion™.

OSIRIS is designed to be flexible for ease of integration with the laboratory's own workflow, including its LIMS and other software, reducing manual intervention in both analysis and data transfer. It increases the efficiency and accuracy of analysis by categorizing artifacts either as critical, requiring human review, or non-critical, dependent on user-designated parameters. Analysis is fast, typically under 30 seconds per 96 well plate. For poor quality samples, OSIRIS can intelligently predict required rework conditions. An audit trail of editing by technicians and reviewers is preserved, and laboratory review procedures can be software enforced. Reporting and data export are very flexible, allowing laboratories to export tables, LIMS-formatted data, and other formats of the laboratory's own design, and can be automated to export subsets of data automatically upon analysis. OSIRIS also has quality metrics that allow it to be used as a lab process quality assurance tool.

[1]Goor RM, Forman Neall L, Hoffman D, Sherry ST, (2011). A mathematical approach to the analysis of multiplex DNA profiles. Bulletin of Mathematical Biology 73(8):1909-31.

OSIRIS Software

- Open source
- Cross-platform
  - Windows
  - Mac
- Displays
  - Stacked graph
  - Table
- Quality analysis
- Reanalysis prediction
- Validated as an expert system

- Flexible User configuration
- Flexible export
  - Table, LIMS, graphical
- Configured for many kits
- Files in both .hid and .fsa format
- Rapid analysis
  - < 30 s/96 samples

Kits

Many kits are preconfigured.
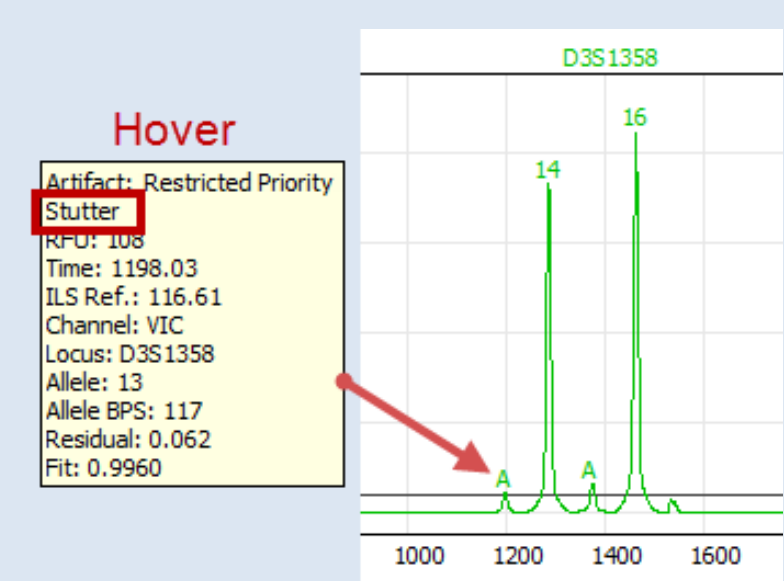Custom multiplexes can be added.
Number of channels not limited.

AB	Promega
Identifiler	PowerPlex 16
GlobalFiler	PowerPlex Fusion
Identifiler Plus	PowerPlex Y
Yfiler	PowerPlex 18D
Profiler Plus	SGM Plus
COfiler	PowerPlex 1.2

Quality analysis

OSIRIS identifies and flags artifacts.   Critical artifacts are flagged for review and acceptance. Non-critical artifacts may be displayed, but do not require review. Artifact display is user-selected.

Critical Artifacts
e.g., Pull-up
flagged for review/acceptance
Hover over flag for more info.
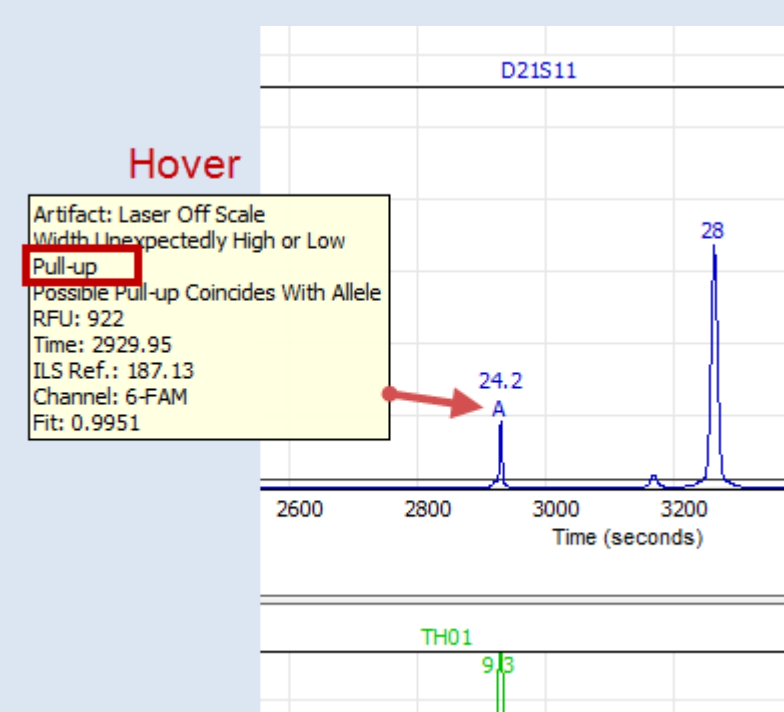
With Critical Artifacts only displayed
(stutter flag not displayed)

Non-critical Artifacts
E.g., Stutter
does not require review.
Hover on displayed flag for info.

With all artifacts displayed

Osiris Uses

- DNA Profile Analysis
- DNA Profile QC
- NDIS Approved Expert System
- CODIS
- Casework
- Relationship testing
- Cell line verification
- Automating Reanalysis
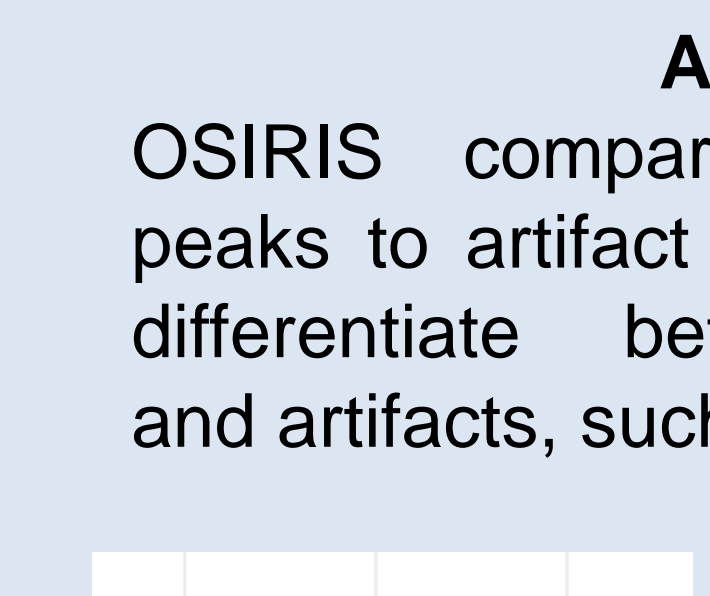- Process monitoring
- Training

Peak analysis

Osiris mathematically analyzes data in .fsa and .hid files, compares the curves to peak, artifact, and baseline signatures, and assesses sample quality with user criteria.
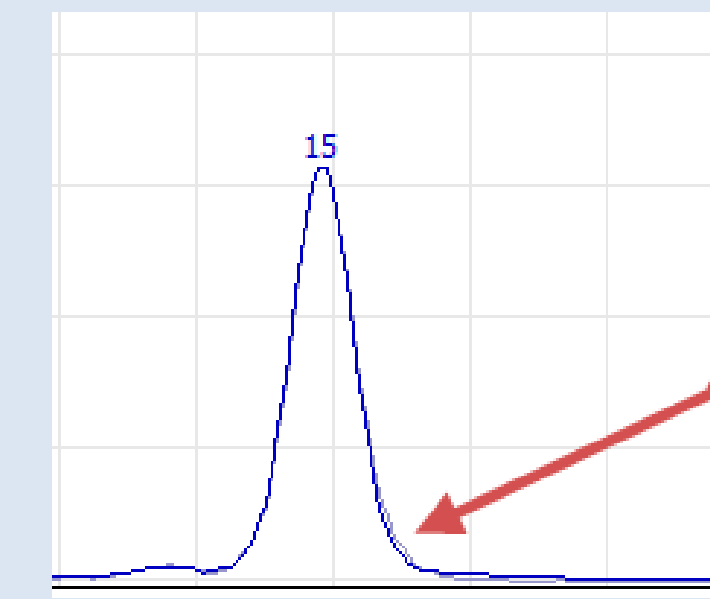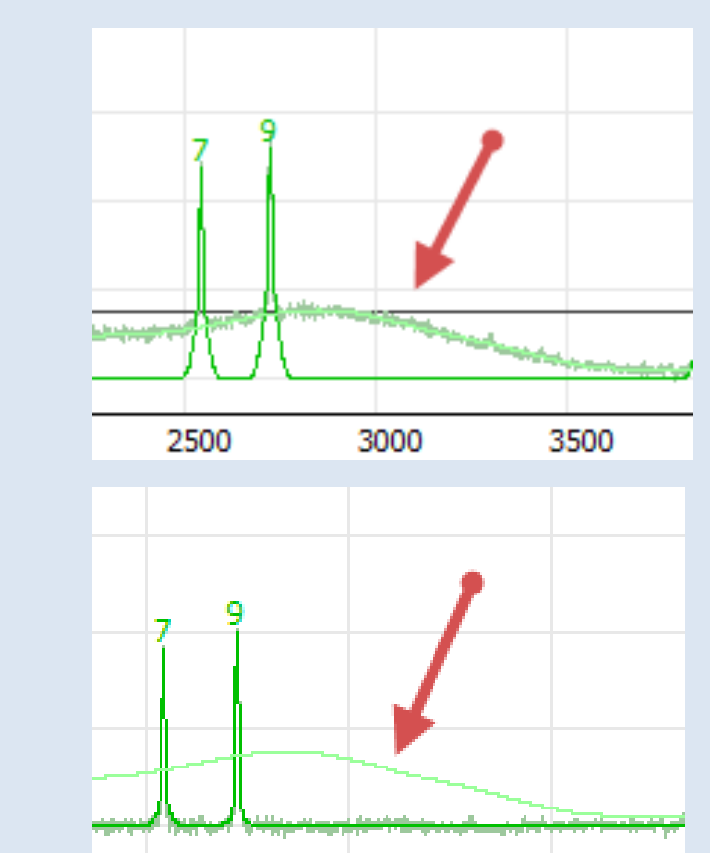

Peak Fitting
The mathematical curve is dark blue, and the raw data is light blue. Fit is excellent, usually better than 0.999.

Artifact Fitting
OSIRIS compares identified peaks to artifact signatures to differentiate between alleles and artifacts, such as pull-up.

Baseline Fitting
OSIRIS can fit a mathematical curve to an elevated baseline (red arrow), allowing it to be subtracted, giving more accurate peak heights at low analysis thresholds.

Artifact recognition

OSIRIS recognizes 150 artifact signatures. Artifact recognition is sensitive and specific.

Peaks
• Peak morphology
• Adenylation
• Pull-up
• Spike
• Crater
• Blob
• Peak shifting
• Interlocus peak
• RFU > max. thresh.
• RFU < analysis thresh.
• Off scale data
Locus
• Stutter
• Heterozygous peak imbalance
• Off-ladder alleles
• Triallele
• Too many Y-STR peaks
• Allele dropout
• RFU < homozygote thresh.
• Locus dropout
• Amel. extraneous peaks
• Amel./Y-STR X or Y missing
• Autosome/Y-STR mixture
Channel
• Peaks between loci
• Raised baseline
• Excessive baseline noise

Sample
• Sample may be a mixture
• Multiple trialleles - mixture
Batch
• Default ls settings overridden
• Positive control(s) missing
• Negative control missing
• Ladder requires review

Control-specific
ILS
• Incorrect number of peaks
• Incorrect spacing
Ladder
• Incorrect number of alleles
• Incorrect spacing
Negative control
• Primer peaks missing
• Contamination – unexpected peaks
• Negative control requires analyst review
Positive control
• Kit positive not found
• Custom lab positive not found
• Positive control allele mismatch

Intelligent Reanalysis Prediction

Uses knowledge base to determine appropriate reanalysis of samples that do not pass QC and recommend more, less or the same amount of DNA for reanalysis.

• Too little DNA
• Too much DNA
• Degraded DNA vs. Too much DNA
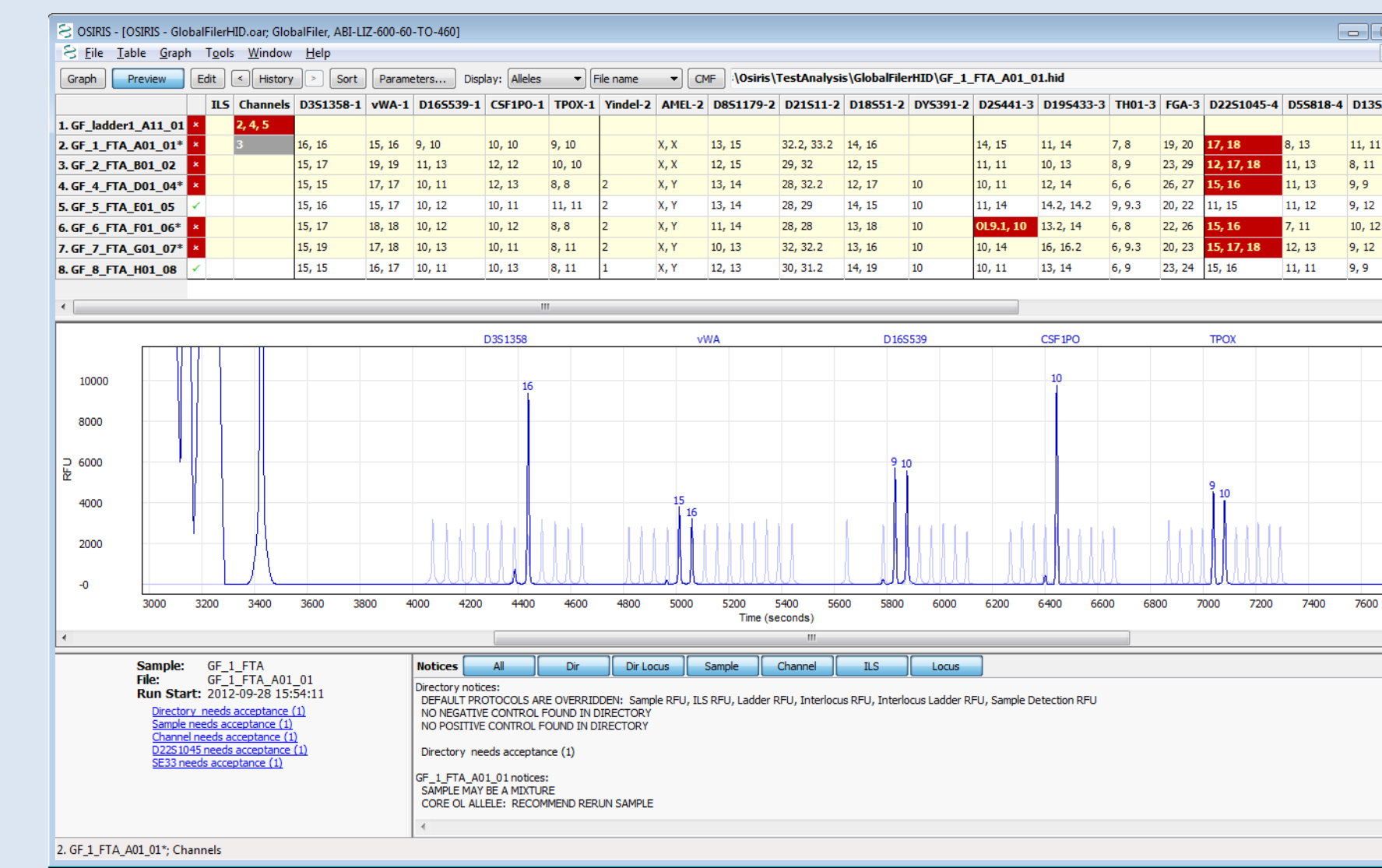• Allows analyst to override automated reanalysis recommendations

Display

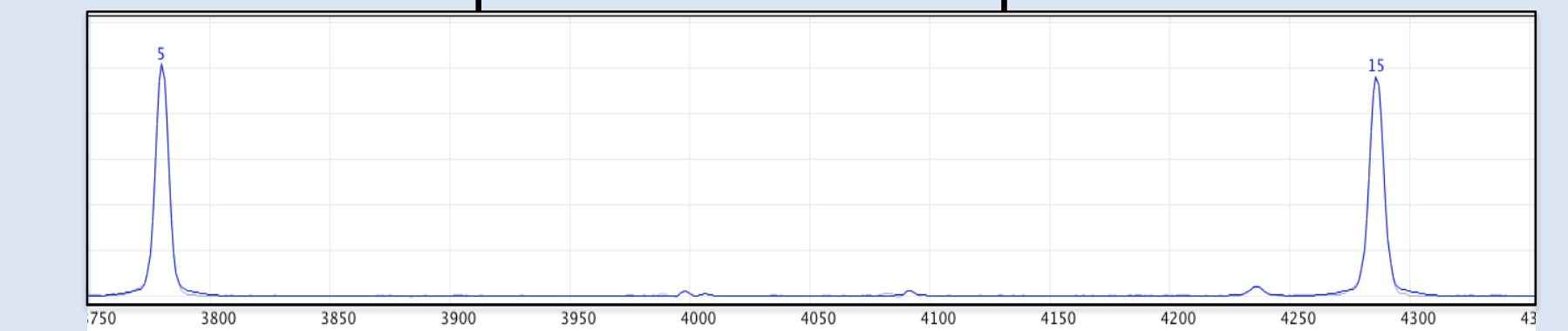Table with preview – GlobalFiler .hid files
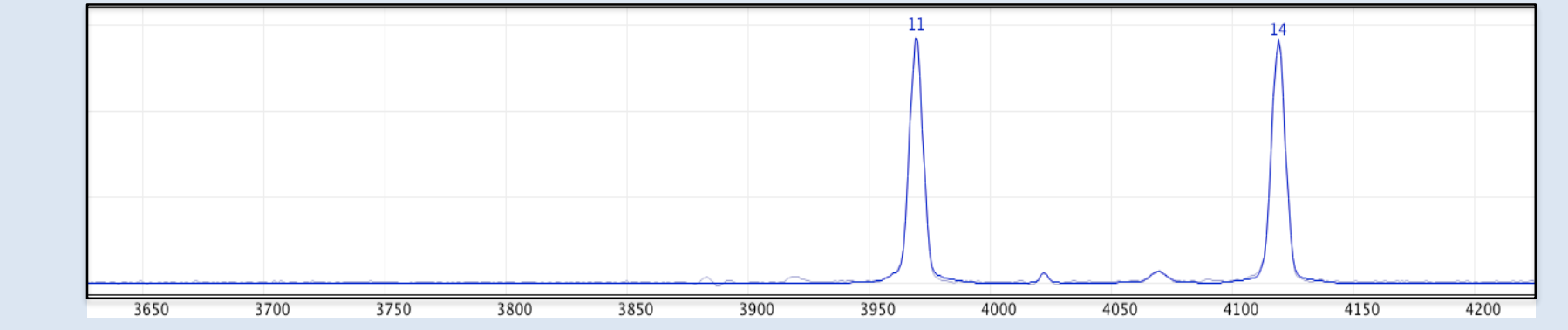

Classical graph view– PowerPlex 16 .fsa files

Using OSIRIS

OSIRIS software is freely available for download on the OSIRIS web page, along with hints for installation. :

http://www.ncbi.nlm.nih.gov/projects/SNP/osiris/

The OSIRIS User's Guide is available on the homepage and in the program itself (F1). It includes a tutorial for a quick start in using the software. The download also includes a variety of demonstration .fsa and .hid format data generated using several different kits.

Open Source Collaboration
OSIRIS source code is currently available for download from the GitHub source code repository.

A number of groups are interested in collaborating on improving OSIRIS. We invite new collaborators to join the OSIRIS community.

For questions or requests, please contact:
forensics@ncbi.nlm.nih.gov

For announcements regarding OSIRIS, please subscribe to the forensics-announce list:
http://www.ncbi.nlm.nih.gov/mailman/listinfo/forensics-announce

Send email.

Home page and contact email.

Chimerism Analysis
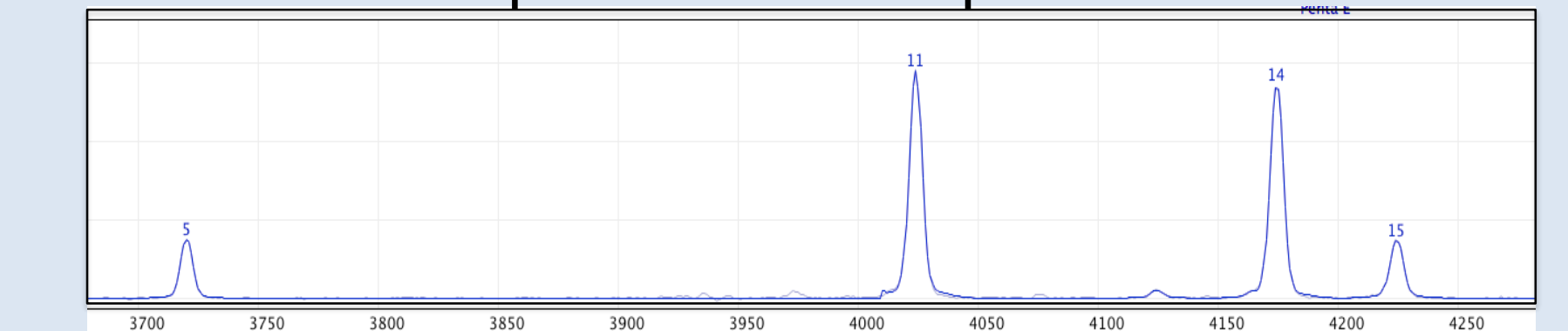
Penta E locus

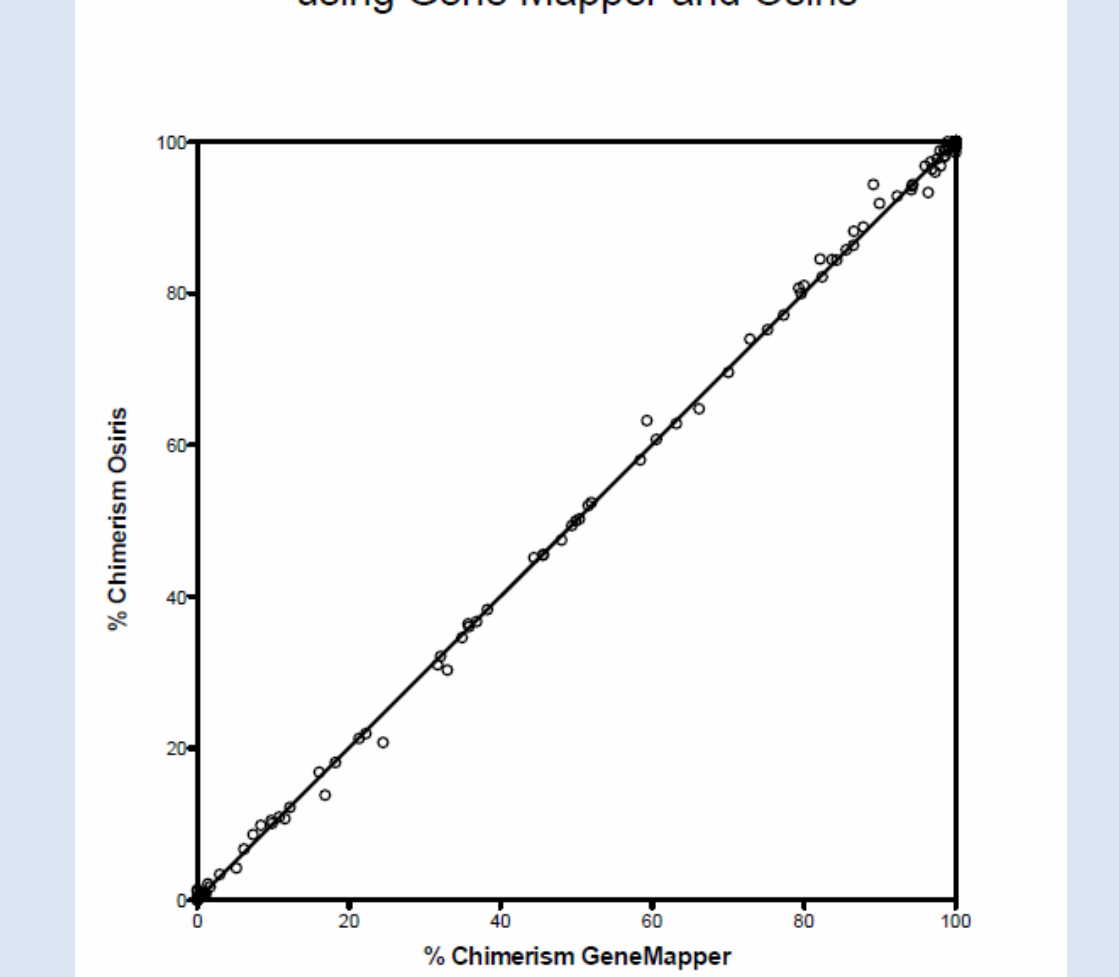Recipient before transplantation

Donor

Recipient after transplantation
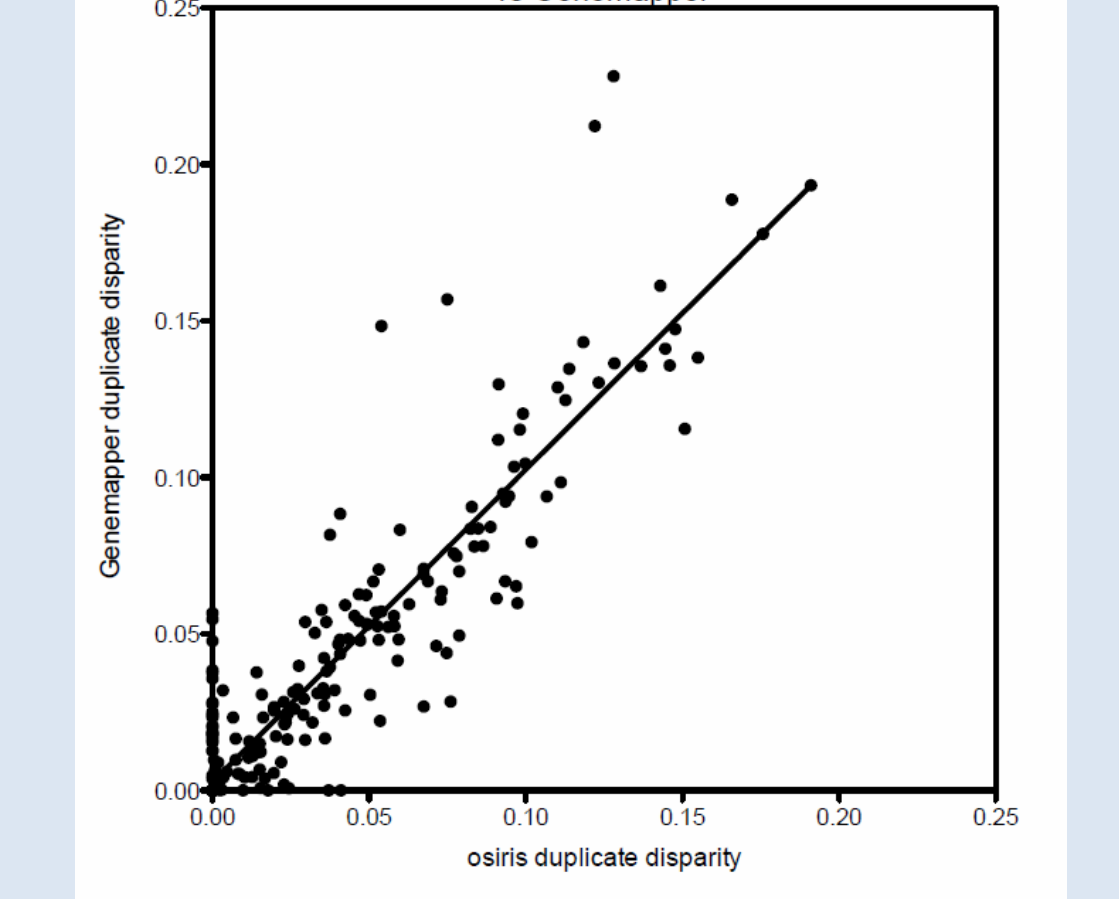

77% Donor Chimerism

Analysis Comparison

Correlation in % chimerism in Quadruplicate values using Gene Mapper and Osiris

Osiris and GeneMapper give similar chimerism analysis results, when the analysis is done using two loci in two PCR replicates of the same sample.

Comparison of Deviation between replicate values with Osiris vs Genemapper

Difference in chimerism estimate of PCR replicates of a single sample analyzed with GeneMapper and with Osiris. Axis distance from the equivalence line shows the degree of disparity between the two programs.

The two graphs suggest that there is significant variability in PCR replicates of the same sample. Variability can be reduced by analyzing multiple loci or PCR replicates