**OSIRIS V2.3 Change Log**

04/23/2014

**Beta 1 Changes** (10/13/2014):

Bug fixes:

1. Corrected code that could crash with non-ASCII format text
2. Changed ILS so that control peaks cannot be restricted priority, which corrected mishandling of fractional filters for ILS peaks
3. Fixed broken link in MessageBook that reported Y allele missing in negative control because no AMEL present

New features:

1. Improved normalization to make more robust: negative peaks are now fit and analyzed to prevent baseline estimation algorithm from sampling pull-down; baseline sample points are added in areas of sparseness for shape control; fit curves with mode near left endpoint considered as baseline instead of peaks; tests are made of peak versus values at left and right to assess if peak is fit to noise; peak standard deviations are converted to ILS base pairs and excessively wide peaks (sigma > 2 bps) are eliminated; raw data samples inconsistent with previous and next samples are removed to eliminate high frequency data
2. Baseline curve added to .plt files along with a button to turn it on/off
3. Added ability to select controls and ladders based on sample name – option selectable in Lab Settings
4. Made inter-locus pull-up non-critical and restricting, so non-critical regardless of other artifacts
5. Create export that compares analyses of same samples injected for 5 secs and for 10 secs, and selects best sample

**Beta 3 Changes** (01/07/2014):

Bug Fixes:

1. Corrected overly restrictive ladder locus spacing safe guards that regulate certain min and max inter-allelic distances
2. Modified code that handles two peaks having same locus allele call so that they are not called craters (with no pull-up). They are now called poor morphology multi-peaks and only one is called – the one with the lowest residual. Removed additional code in cross channel analysis that created craters based solely on inter-peak spacing.
3. Changed adenylation analysis to look only for –A.
4. Eliminated “Excessive Noise” message for ILS’s. Either ILS analysis succeeds or it doesn’t.
5. Fixed “Unexpected Peaks in Neg Ctrl…” message so that peak location is given in ILS-base pairs instead of locus base pairs.
6. Changed message when laser off-scale but no pull-up was found to say “Laser Off Scale: No Pull-up Identified”.
7. Stutter tests are no longer performed for an ILS.
8. Corrected MessageBook messages that referred to “inter-locus” peaks when “extended locus” peaks were intended.
9. Corrected bug in ladder locus allele identification to prevent a locus from capturing peaks that actually belong to the locus immediately to the left. Now, each locus analysis disregards peaks that are already labelled as control peaks, as well as any peaks to the left of control peaks.
10. For building under Linux, added “#include <stdlib.h> to rgstringdata.cpp in the Tools library.
11. Found and eliminated spurious 1 sec analyzed peaks. These occurred during the building of the overall analyzed curve by double sampling adjacent peaks under extremely rare circumstances.
12. Corrected MessageBook ordering error that caused craters in the inter-locus to be labelled incorrectly. Also, messages that are set to be evaluated in the MessageBook must not be set by the software. Craters share messages with side peaks, but these messages must be measure messages only.

New Features:

1. Added preset message to MessageBook and software access to allow user to set window width for dynamic baseline estimation. Default changed from 9 to 15.
2. Baseline estimation uses raw data for which analyzed data is below a threshold. Changed threshold from hard coded percentage of minRFU to a user specified RFU threshold. Default is 10 RFU.
3. Baseline estimation for normalization improved in high noise situations by optionally prefiltering raw data using a user specified filter window (default = 15). Once baseline is estimated, it is subtracted from original raw data (filtered data is deleted) and then analyzed. (Does not apply to non-normalization baseline estimation.)
4. Added ability to estimate the quality of the sample-to ladder fit using the metric formed by evaluating the cubic spline time transform from sample space to ladder space and calculating the maximum second derivative. This measures the linearity of the map. Added optional test and threshold to warn if metric is “too large”.
5. Added ability to optionally suggest rework if too many excessive residuals in sample.
6. Added ILS’s for Identifiler and IdentifilerPlus that optionally omit the ILS 340 peak or the ILS 250 and ILS 340 peaks. Added ILS for PowerPlex 16 that omits the ILS 225 peak.

**Beta 4 Changes** (02/08/2014):

Bug Fixes:

1. Corrected array overrun in baseline normalization, where the algorithm was reading raw data values beyond the end of the raw data array.

New Features:

1. Cross channel analysis has been rewritten to be more reliable and more robust. A new type of phenomenon has been incorporated, called sigmoidal peaks that have a positive and negative component, and negative peaks are analyzed as well. All spacing is now based on ILS-base pairs and not on time. Based on spacing, potential craters and sigmoids are assessed at the start and eliminated if they do not arise from a pull-up. No peak can be considered a primary pull-up unless its height exceeds a user-specified threshold. Pull-ups are assessed based on peak proximity differences measured in ILS-base pairs and reflect peak types. No crater peak can also be assessed as a side peak to a different crater.
2. A message has been added that allows the user to specify in Lab Settings the minimum height for a primary pull-up.
3. The multi-peak poor morphology message has been made non-critical and such peaks are not called as alleles.
4. Improved baseline estimation for normalization when prefiltering by adding sample points more often than when not filtering and also adding more points for shaping. This is because filtering spreads peaks and reduces the size of intervals available for baseline sampling.

**Beta 5 Changes** (03/17/2014):

Bug Fixes:

None

New Features:

1. Added PowerPlex ESX 16 and 17 and PowerPlex ESI 16 and 17 marker sets.
2. Because problems arose with the ladder analysis of the SE-33 locus in ESX 17, namely, there were 37 required peaks to select from 67 candidates, rewrote ladder locus analysis algorithm using known ILS base pair information to more easily select correct peaks. Resulting algorithm is O (n2) while the old algorithm was O (n!). The new algorithm is also more robust and improves peak selection for evenly spaced ladder loci.
3. Added ability to distinguish alleles from artifacts based on excessive residual displacement (ERD) with each locus. The test requires the selection of a “known” valid allele in a locus, typically the tallest allele, and then calculates the difference between the residuals of each other candidate allele in the locus and the residual of the valid allele – the residual displacements. Displacements in excess of the user-specified threshold (default = 0.17 base pair) indicate an unlikely allele. ERD artifact peaks are not called as alleles, but they can be edited to override the software recommendation.

**Beta 6 Changes** (04/01/2014):

Bug Fixes:

None

New Features:

1. Based on experience with more samples, changed ERD test so that ERD only reports in the presence of one or more secondary conditions from the list: pull-up, laser off scale, unacceptable fit, marginal fit, spike, blob, width unexpected large or small, or multi-peak morphology. If the user specifies that the ERD artifact should be non-critical, then the peak is non-critical independent of other artifacts.

**Beta 7 Changes** (04/07/2014):

Bug Fixes:

1. Corrected (rare) situation in which a negative peak adjacent to any positive peak and coinciding with a peak in another channel can be called sigmoidal pull-up. Now, to be identified as a sigmoid, the positive peak can be no more than 3 times the height of the negative one.
2. Corrected situation in which some peaks report having zero area. This occurred because only double Gaussian peaks calculated their own areas. Added the calculation to Gaussian and super Gaussian curves, which, currently, are the only other peaks for which an area calculation can be performed.

New Features:

1. Based on experience with samples, removed pull-up and laser off-scale from acceptable secondary conditions list for ERD.

**Beta 8 Changes** (04/20/2014):

Bug Fixes:

None

New Features:

1. Added capability to distinguish between known single source samples and possible mixtures based on user-specified file name/sample name search strings. Using Lab Settings presets, the user can choose to disable low level height filters for possible mixtures. The user can choose to disable one or more of the following: fractional filter, pull-up fractional filter, stutter filter (positive and negative), and adenylation filter. The specified sample analysis and detection thresholds still apply uniformly across all samples.