Raised Baseline Options:

Raised Baseline Threshold for Samples (RFU) 250

Raised Baseline Threshold for Sample ILS Channels (RFU) 250

**Raised Baseline Threshold for Samples (RFU)** and **for Sample ILS Channels (RFU)** parameter sets the threshold for raised baseline detection in the allele channels and the ILS channel respectively. Raised baseline can result in minor peaks either not being called or appearing to have a higher RFU than they should actually have. Needs advice and impact here.

Cross Channel Options:

Attempt to Apply Embedded Color Correction Matrix 

Min RFU for a peak to be considered as a primary pull-up (Default = 500) 500

Make Pull-up At Allele Artifact Non-Critical 

**Attempt to apply Embedded Color Correction Matrix** causes OSIRIS to apply the color matrix to .fsa files where it has not already been applied (some Applied Biosystems 310 Genetic Analyzer files). These files appear to have extreme pull-up, and will fail to analyze correctly without application of the matrix. Files without an embedded color matrix, where the color matrix has already been applied, such as those produced by the 3100 and higher Genetic Analyzers, will be unaffected if this parameter is set. However, if an Applied Biosystems 310 Genetic Analyzer file has had the color matrix applied and also has an embedded matrix analysis, it will be adversely affected by reapplication of the matrix with this parameter. Most files requiring this were produced with out of use procedures/software. Unless files are known to require this parameter it should be unchecked by default.

**Min RFU for a peak to be considered as a primary pull-up (Default = 500)** is the minimum height at which OSIRIS will consider a peak to be the cause of pull-up (primary pull-up) in other channels. This helps rule out pull-up where low level peaks in different channels simply coincide.

**Make Pull-up at Allele Artifact Non-Critical** setting determines whether “Pull-up at Allele” will be reported as a critical (unchecked) or non-critical artifact. Typically, the Pull-up at Allele artifact occurs when actual allele peaks in different channels comigrate. Users that wish to be notified if alleles in different channels comigrate should uncheck this setting.

Enable Test for Excessive Noise 

Test for Excessive Noise Above Analysis Threshold (checked) or below (unchecked) 

**Enable Test for Excessive Noise** tests for excessive noise in the baseline. Excessive noise in the baseline can result in minor peaks not being called. See the explanation of [excessive noise](#Excessivenoise) in the artifact handling section. What is the impact on not setting this without normalization, with normalization?

**Test for Excessive Noise Above Analysis Threshold (Checked) or Below (Unchecked)** causes OSIRIS to flag samples when excessive baseline noise occurs, including below the analysis threshold (but above detection threshold), or only when it occurs above the analysis threshold. This setting is active only if the excessive noise test is activated (above). What happens if this is unchecked and the detection and analysis thresholds are the same?

Flag Mixed Samples and Triallelic Loci 

**Flag Mixed Samples and Triallelic Loci** causes OSIRIS to flag mixed samples and triallelic loci. Samples with one or more loci with four or more alleles or that have a number of loci with trialleles that exceeds the threshold set for mixed loci will be flagged as mixed samples. This setting also flags triallelic loci that are not listed as accepted trialleles.

Rework Options:

Recommend Amp More On Low Homozygote 

Select Reamp Regular (checked) Versus Reinject (unchecked) 

Recommend Rework If Laser Off Scale Found 

These settings affect how Osiris predicts that samples might be reanalyzed to obtain a result with fewer artifacts. These predictions can be exported and could be used in a high-throughput laboratory to help automate reanalysis of failed samples.

**Recommend Amp More on Low Homozygote** causes OSIRIS to flag samples with a homozygote peak below the acceptable homozygote threshold or with a homozygote that has a peak between analysis and detection thresholds that is not a known artifact (stutter, etc.) for reamplification with more DNA.

**Select Reamp Regular (Checked) vs. Reinject (Unchecked)** determines whether OSIRIS will flag samples needing rework for reinjection or reamplification. The setting of this parameter will depend on whether the laboratory process is designed with reinjection or reamplification in mind.

**Recommend Rework if Laser Off Scale Found** will cause OSIRIS to flag samples with off-scale data for reamplification with less DNA.

Curve Fit Options:

Ignore noise analysis in peak detection when above detection threshold 

Percentage of Standard Noise Threshold for Peak Identification (Default = 100) 100

**Ignore noise analysis in peak detection when above detection threshold** causes OSIRIS to detect peaks above the detection threshold even in the presence of noise. We suggest selecting this parameter unless the user sees an excessive number of insignificant peaks detected. Checking this parameter may be beneficial when using OSIRIS with very low analysis or detection thresholds (below 75). This parameter is probably unnecessary for analysis and detection thresholds greater than or equal to 100 RFU, or if baseline normalization is specified. (See below).

**Percentage of Standard Noise Threshold For Peak Identification (Default = 100)** adjusts the sensitivity of peak detection. This allows the user to reset the threshold for testing locally averaged raw data to determine if there is sufficient area under the curve for a peak to be fit. Setting this to a low value, such as 10-25, causes OSIRIS to be more sensitive to low-level peaks. Setting the value higher than 100 causes OSIRIS to ignore more low-level peaks. The standard noise threshold is analysis platform specific, to account for different analyzer sensitivity, which results in different levels of noise. Are we taking the platform into account automatically or does the user have to do that?

Negative Control Options:

Test for Primer Dimer Peaks in Negative Controls 

Minimum Height for Primer Dimer Peaks (RFU) 2000

Minimum Number of Peaks per Channel in Primer Dimer for Negative Control 2

Test for Presence of Sub-Analytic Peaks in Negative Controls 

**Test for Primer Dimer Peaks in Negative Controls** causes OSIRIS to perform the test for primer peaks in negative control samples. When unchecked the test is not performed.

**Minimum Height for Primer Dimer Peaks (RFU)** sets the minimum threshold for identifying primer peaks in the negative control. This threshold can be empirically determined by the laboratory. It should be set well above the height of any pull-up peaks caused by the internal lane standard channel, generally close to, but below the maximum threshold intensity for the detection platform.

**Minimum Number of Peaks per Channel in Primer Dimer for Negative Control** helps to discriminate the primer peaks in the negative control. This should be set to at least two peaks.

**Test for Presence of Sub-Analytic Peaks in Negative Controls** will cause negative controls to be flagged if there are peaks whose RFU is below the analysis threshold, but whose RFU is greater than the detection threshold. Peaks in the negative control that fall in the range between the detection and analysis thresholds may indicate low level contamination before it becomes an issue in the laboratory. This has no effect if the detection threshold is not set.

Apply Fractional Filter To Peaks Below Analysis Threshold (Homozygous Loci) 

**Apply Fractional Filter to Peaks Below Analysis Threshold** causes OSIRIS to apply the fractional filter (if set) to peaks below the analysis threshold and above the detection threshold in homozygous loci. If this is left unchecked, the fractional filter will not be applied to peaks below the analysis threshold, but will be applied (as set) to peaks above the analysis threshold. Note that this case can result in a situation in an apparently homozygous locus where a second peak that lies between the analysis and detection threshold would trigger a notification, but a second peak peak slightly above the analysis threshold would not, because it was filtered out by the user-defined fractional filter settings.

Call Criteria:

Do Not Call OL Crater If Laser In Scale and Raised Baseline 

Do Not Call OL Allele If Pull-up 

Do Not Call OL Alleles If Excessive Number of OL’s 

Call Peaks That Are Identified as Adenylation If On-Ladder 

Do Not Call Alleles With Excess Residual 

**Do Not Call OL Crater If Laser In Scale and Raised Baseline** reduces the number of small crater artifacts that OSIRIS calls and that require subsequent editing. This setting causes OSIRIS to not call a crater as an allele if: there is no off-scale data at the artifact location; there is raised baseline in the artifact channel; and if the artifact is off-ladder. Very small crater artifacts can be called when the analysis threshold is set low enough that a raised raw data base line is above or even near the analysis threshold. Since there is no off-scale data at this artifact location, pull-up is unlikely in this situation. Selecting this parameter will reduce the number of small crater critical artifacts also called as alleles. Editing will allow the allele call to be re-applied to the peak.

**Do Not Call OL Allele If Pull-up** causes OSIRIS to not mark with an allele call those peaks that are flagged as both pull-up and off-ladder. Selecting this parameter will reduce the number of allele calls on pull-up artifacts, but does not affect flagging the artifact and artifact notification. This may reduce the amount of editing required to remove pull-up allele calls from the graph and table. Editing will allow the allele call to be re-applied to the peak.

**Do Not Call OL Alleles If Excessive Number of OL’s** causes OSIRIS to not mark with an allele those peaks that are off-ladder if the “Max. off-ladder alleles per sample” threshold is exceeded. Selecting this parameter may reduce the number of pull-up or shifted peaks that are labeled with an allele. However, if this rule is triggered, genuine off-ladder alleles will also not be labeled. Editing will allow any affected allele calls to be re-applied to their peaks.

**Call Peaks That Are Identified As Adenylation If On-Ladder** causes OSIRIS to call peaks as alleles if they are on-ladder or “accepted” alleles. For example, when this parameter is selected, in a mixture where there is a major contributor allele 9, a minor 8.3 peak that was below the adenylation threshold would be called as an 8.3 allele if there is an 8.3 ladder allele or if the 8.3 allele is indicated as an accepted allele in the Off-ladder alleles list on the Assignments tab in the Lab Settings. This will also ensure accurate calling of the minor TH01 9.3 allele when it is below the adenylation threshold in a TH01 9.3/10 mixture. Laboratories analyzing mixtures should select this setting. Will the TH01 9.3 be called if 9.3/10 has 9.3 below the adenylation threshold, if this is unchecked?

**Do not call alleles with excess residuals** causes OSIRIS to not call an allele for any peak flagged as having excess residual. Selecting this parameter can reduce the number of allele calls on artifactual peaks such as those with shifting. Editing will allow the allele call to be re-applied to the peak. See the description of Excessive Residual below

Do Not Report Heterozygous Imbalance If Sample Is Mixture 

**Do Not Report Heterozygous Imbalance If Sample Is A Mixture** causes OSIRIS to not trigger a heterozygous imbalance notice in any locus in a sample if the sample meets the conditions to be flagged as a mixture.

Baseline analysis is more fully described in Appendix H: Dynamic Baseline Analysis.

Baseline Analysis Options:

Test Adjusted Signal Heights Relative To Baseline (Overridden by below) 

Normalize Raw Data Relative to Baseline (Overrides above) 

Baseline Estimation Threshold (In RFU; Must Be Positive; Default = 10 RFU) 10

Ignore Effects of Negative Relative Baseline 

Enable Raw Data Filter For Baseline Normalization Estimation 

Select Triple Pass Filter (Checked)(Preferred) or Single Pass Filter (Unch) 

Triple Pass Filter Window Width for Baseline Est. (Def. = 7) 7

Single Pass Filter Window Width for Baseline Est. (Def. = 15) 15

**Test Adjusted Signal Heights Relative To Baseline (Overridden by below)** causes OSIRIS to calculate a dynamic baseline but not to normalize the raw data. Instead, OSIRIS uses the dynamic baseline to calculate a relative peak height for each peak. The relative peak heights are used to reassess three potential artifacts: Below Analysis Threshold, Stutter and Adenylation. If a peak has any of those three artifacts before calculation of the relative heights, then the relative height is irrelevant, the peak is given a non-critical artifact, and is not called as an allele. However, if none of those three conditions holds for the absolute peak height, they are each reassessed using the peak’s relative height. If any of the three conditions hold for the relative height, the peak is not called as an allele and is given a critical artifact. Because this new artifact is critical, the peak can be edited by the user. The effect is to calculate peak heights corrected for the baseline and use those corrected peak heights in artifact determination. This preset can be used to evaluate the baseline curve predicted by OSIRIS by displaying the baseline and the raw data simultaneously in the Graph View (“R” and “B” buttons). If the next preset below (“Normalize Raw Data…”) is selected, this preset is ignored by OSIRIS.

**Normalize Raw Data Relative to Baseline (Overrides above)** Baseline normalization eliminates many of the artifacts associated with raised baseline by calculating the raw data baseline and subtracting it from the raw data. Baseline normalization also significantly improves the accuracy of allele/artifact identification and peak heights when analyzing with a low analytical threshold (e.g., below 150 on files produced with the 3100 Genetic Analyzer). Selecting this preset causes OSIRIS to calculate a dynamic baseline and normalize the raw data with respect to it. Peaks are refit with respect to the new raw data and the usual artifacts are called. The critical artifact notices triggered by the “**Test Adjusted Signal Heights**…” parameter are not used with this option. Selecting this parameter causes “**Test Adjusted Signal Heights**…” to be ignored and the critical artifact notices not to be triggered by that option. The effect is to subtract the calculated dynamic baseline from the raw data before detecting alleles and artifacts.

**Baseline Estimation Threshold (In RFU; 1+; Default larger than 5 RFU)** is the height below which analyzed fit curves will be considered to be zero for the purpose of delineating raw data baseline sample intervals. Extremely low values restrict baseline sample intervals (peaks generally have to be further apart). The effect of this is (WHAT’S THE EFFECT?). Extremely high values may give inaccurate baseline sampling data, because very small peaks may be considered to be part of the baseline. The effect of this could be to over-subtract the baseline from the raw data, making some peaks smaller than they should be.

**Ignore Effects of Negative Relative Baseline** – this preset applies to either of the options, “**Test Adjusted Signal Heights**…” (1) or “**Normalize Raw Data…**” (2) When this is selected, a negative baseline is treated as if it is 0. When this is unselected, the dynamic baseline is subtracted either from affected peaks (1) or the raw data (2), whether its value is positive or negative. If neither preset 1 nor preset 2 is set to “true”, then the setting of this preset is irrelevant to Osiris operations. Default = Selected (true). The effect of this could be to make peaks that are situated in the “dip” shorter than they are in reality.

**Enable Raw Data Filter For Baseline Normalization Estimation** – this preset applies only to the option “**Normalize Raw Data…**” above. It instructs Osiris to filter the raw data before sampling in order to improve baseline estimates, especially in the presence of high noise. Once the baseline is estimated, the filtered raw data is discarded. The baseline is subtracted from the original raw data and the resulting normalized raw data is input to the analysis algorithms. Default = Selected (true). WHAT IS THE BENEFIT OF THIS? WHEN SHOULD IT BE/NOT BE USED?

**Select Triple Pass Filter (checked)(preferred) or Single Pass Filter (unchecked)** – the single pass filter uses a single window width for performing a moving average, which is equivalent to applying a low-pass filter to the raw data. In most cases, the single pass filter is satisfactory, but, occasionally, it can introduce a high-frequency artifact into the result. The triple pass filter performs a single pass filter three times with three different window widths and is known to prevent the introduction of a high-frequency component into the result. Therefore, the triple pass filter is preferred. IS THERE A SITUATION WHERE SINGLE PASS WOULD BE PREFERRED?

**Triple Pass Filter Window Width for Baseline Estimation (Default = 7)** – the number of raw data sample points used for the first of three passes in a centered averaging filter to smooth raw data prior to baseline estimation. If applied, this filter is only used to identify the baseline. It does not modify the raw peak data used for analysis. EFFECT OF CHANGING THIS?

**Single Pass Filter Window Width for Baseline Estimation (Default = 15)** is the number of raw data sample points used for a centered averaging filter to smooth raw data prior to baseline estimation. If applied, this filter is only used to identify the baseline. It does not modify the raw peak data used for analysis. EFFECT OF CHANGING THIS?

Ladder Selection Criteria (Based on Sample-to-Ladder Time Transform):

Select Most Linear Time Transform (Checked) or Lowest Error Time Transform (Unch.) 

Enable Ladder Fit Threshold Test For Accurate Sizing 

Most Linear Time Transform Threshold (Default = 175, 0 is ideal fit) 175

Least Time Transform Error Threshold (Default = 35%, 0 is ideal fit) 35

**Select Most Linear Time Transform (Checked) or Lowest Error Time Transform (Unchecked)** – the time transform from sample to ladder that maps sample alleles into the ladder time frame can be tested for accuracy in multiple ways. If the transform is linear, then it will also be perfectly accurate, but the reverse is not true. An accurate transform does not have to be linear. The most linear time transform yields the least peak distortion and, most often, yields the same ladder selection as the least error transform. WHICH SHOULD THEY CHOOSE?

**Enable Ladder Fit Threshold Test** – this preset causes Osiris to calculate and test the sample-to-ladder fit metric. The specific metric depends on the selection above. If the “most linear” selection is made above, then the metric is related to the maximum second derivative of the transform. If the “least error” selection is made above, then the metric is based on an error estimation formula which incorporates higher order derivatives and the maximum interpolation interval. In either case, if the value exceeds the appropriate threshold specified below, then the sample receives a critical artifact warning that allele calls may not be reliable. Default = Selected (true).

**Most Linear Time Transform Threshold (Default = 175, 0 is ideal fit)** is a metric for measuring compatibility between (chosen) ladder and sample, based on the maximum second derivative of the time transform. Above approximately 175, calls may be inaccurate, resulting in off-ladder calls.

**Least Time Transform Error Threshold (Default = 35%, 0 is ideal fit)** – is a metric for measuring compatibility between (chosen) ladder and sample, based on the maximum error of the time transform in base pairs. Above approximately 35% of a base pair, calls may be inaccurate, resulting in off-ladder calls.

Enable Residual Displacement Allele Validation Test 

Max % BP for Residual Displacement Test (Default = 17% BP) 17

Make Excessive Residual Displacement Message Critical 

“Residual” is the migration difference between the sample and ladder peaks, sometimes called shifting. Residual Displacement is the difference in Residual between two peaks in a locus.

**Enable Residual Displacement Allele Validation Test** – This can reduce the amount of editing required by reducing the number of artifacts that receive an allele call. This setting causes OSIRIS to perform the residual displacement allele validation test using the %BP threshold specified above. This test, which operates independently within each locus, measures the displacement of each locus peak residual from the residual of a calculated valid locus peak. Excessive residual displacement indicates a peak that did not migrate with the true allelic peaks. Such peaks are marked with an artifact and, if reported, are not called as alleles. However, the artifact is only reported if one or more of the following artifacts is also detected for the peak: curve fit marginal, curve fit unacceptable, spike, blob, width unexpectedly high or low, or crater or poor morphology. Default = Unselected (false). This helps to reduce the number of artifact peaks that receive an allele call. The user may re-enable the allele call on those uncalled peaks.

**Max % BP for Residual Displacement Test (Default = 17% BP)** is the acceptable size difference between the residuals of a known valid peak in a locus and any other locus peak. Peaks with excessive residual displacement will not be called as alleles. A known valid peak is either the tallest peak or the next tallest (if at least half the height of the tallest) subject to the absence of certain artifacts, including pull-up and unacceptable curve fit.

**Make Excessive Residual Displacement Message Critical** – this preset applies only if the residual displacement test is enabled. See above. If selected, a peak with an excessive residual displacement is given a critical artifact. If not selected, the artifact is non-critical. Default = Unselected (false).

Disable Low Level Height Filters For Known Mixtures 

Disable Fractional Filter 

Disable Pull-up Fractional Filter 

Disable Stutter Filter 

Disable Adenylation Filter 

**Disable Low Level Height Filters For Known Mixtures** – this preset can be used to distinguish between single source and potentially mixed samples, so that they can be differentially analyzed. This allows use of filters for single source samples that may not be appropriate for use with mixed samples. This may be particularly useful when using OSIRIS output for performing computer-assisted mixture analysis. When checked, the options selected below (fractional filter, pull-up fractional filter, stutter filter, and adenylation filter) are disabled for samples that are specified as possible mixtures, as determined by substrings within either the file name or sample name (similar to identification of positive and negative controls). The identifying substrings are specified in the Lab Settings under the File/Sample Names tab. Samples can be identified in one of two ways. The “Possible Mixture” category substrings can be used to identify all samples that are potential mixtures, or the “Single Source” category can be used to identify all samples that are known to be single source (i.e., reference or comparison samples). If both are specified, only the Single Source substrings are actually used. Positive and negative controls are automatically considered to be single source. Default = Unselected (false).

**Disable Fractional Filter** – if selected, the fractional filter is disabled for specified potential mixtures. This preset applies only if **Disable Low Level Height Filters For Known Mixtures** is selected. See above.

Default = Unselected (false).

**Disable Pull-up Fractional Filter** – if selected, the pull-up fractional filter is disabled for specified potential mixtures. This preset applies only if **Disable Low Level Height Filters For Known Mixtures** is selected. See above. Default = Unselected (false).

**Disable Stutter Filter** – if selected, the stutter filter is disabled for specified potential mixtures. This preset applies only if **Disable Low Level Height Filters For Known Mixtures** is selected. See above.

Default = Unselected (false).

**Disable Adenylation Filter** – if selected, the adenylation filter is disabled for specified potential mixtures. This preset applies only if **Disable Low Level Height Filters For Known Mixtures** is selected. See above. Default = Unselected (false)