**How to Run OSIRIS from a Command Line**

The collection of strings can be typed into a file with any name. For illustration, I’ll use BaseInput.txt. Put BaseInput.txt into the same directory as the Osiris installation. That is the directory that contains the program: TestAnalysisDirectoryLC.exe. This is the program that runs the analysis. All of its outputs are in text format. To view them using Osiris, you would have to copy them to either a Windows or Macintosh environment and use the Osiris installation there.

The command (at least on Windows) would then be:

TestAnalysisDirectoryLC.exe < BaseInput.txt

which causes the contents of BaseInput.txt to be piped to the standard input and be available for TestAnalysisDirectoryLC.exe to read.

**Input Strings (actual order of lines is unimportant, but each line must end in a semicolon, with last line a lone semicolon):**

1. InputDirectory = *name*;
2. LadderDirectory = *name*;
3. ReportDirectory = *name*;
4. OutputSubdirectory = *name*; (optional)
5. MarkerSetName = *name*;
6. LaneStandardName = *name*;
7. CriticalOutputLevel = *number*;
8. MinSampleRFU = *number*;
9. MinLaneStandardRFU = *number*;
10. MinLadderRFU = *number*;
11. MinInterlocusRFU = *number*;
12. RawDataString = *character*; (‘a’ or ‘A’ means “analyzed”; anything else means “raw”)
13. SampleDetectionThreshold = *number*;
14. AnalysisThresholdOverride:*channel\_number* = *number*;
15. DetectionThresholdOverride:*channel\_number* = *number*;
16. FullPathToMessageBook = *name*;
17. FullPathToLabAndStdSettings = *name*;
18. At the end, a line with nothing on it but a “;” will cause Osiris to stop reading.

Here is an example:

InputDirectory = C:/Apps/User\_Installs/NCBI/osiris/TestAnalysis/Identifiler/Identifiler\_Artifacts;

LadderDirectory = C:/Apps/User\_Installs/NCBI/osiris/Config;

ReportDirectory = C:/Users/goorrob/Documents/Osiris;

MarkerSetName = IdentifilerPlus Less ILS250;

LaneStandardName = ABI-LIZ-LESS250and340-500;

CriticalOutputLevel = 15;

StandardSettings = C:/Apps/User\_Installs/NCBI/osiris/site/Volumes/V-20160201-115334/V-20160201-115334\_StdSettings.xml;

LabSettings = C:/Apps/User\_Installs/NCBI/osiris/site/Volumes/V-20160201-115334/V-20160201-115334\_LabSettings.xml;

MessageBook = C:/Apps/User\_Installs/NCBI/osiris/Config/LadderSpecifications/MessageBook.xml;

MinSampleRFU = 24;

MinLaneStandardRFU = 100;

MinLadderRFU = 75;

MinInterlocusRFU = 24;

MinLadderInterlocusRFU = 75;

SampleDetectionThreshold = 10;

RawDataString = R;

;

Explanations: (each separate input string is to be entered on one line, with no line feed)

1. InputDirectory is the directory where your input (.fsa or .hid) files are located.
2. LadderDirectory is the location within the Osiris installation of all of the ladder files. It will always have the form …/Osiris/Config
3. ReportDirectory is the location where you choose to put your output files. Osiris will create a subdirectory with name based on the name of your Input Directory.
4. MarkerSetName is the name of the marker set to be used for your analysis (see note below)
5. LaneStandardName is the name of the lane standard to be used for your analysis (see note below)
6. CriticalOutputLevel is the message level below which Osiris calls the message critical. 15 is the default value.
7. StandardSettings is the full path name of the standard settings file. This will be the standard settings file in the custom (or default) operating procedure you are using. This OP is always located within your Osiris installation. If it is a custom OP, the path will always begin with: …/Osiris/site/Volumes/ and the file name will always end with \_StdSettings.xml. The “V” name above (V-20160201-115334) is coded to contain the time and date of creation of the OP and is assigned by Osiris at that time.
8. LabSettings is the full path name of the lab settings file for the operating procedure you are using. As in (7), this file is located within your Osiris installation. If you are using a custom OP, the path will always begin with: …/Osiris/site/Volumes/ and the file name will always end with \_LabSettings.xml.
9. MessageBook is the full path name of the MessageBook file that Osiris uses as part of its logical analysis engine. There is a centralized MessageBook that is used for all kits and for all analyses. It is located in your Osiris installation, so the location is

…/Osiris/Config/LadderSpecifications/MessageBook.xml

1. MinSampleRFU is the default minimum RFU for calling an allele on any sample channel (does not apply to lane standard). To override this, say, for channel 1, requires an additional line:

AnalysisThresholdOverride:1 = 50;

For the example, this would cause the default channel value of 24 to be overridden by the value 50 for channel 1.

1. MinLaneStandardRFU is the minimum RFU for lane standards.
2. MinLadderRFU is the minimum RFU for ladder channels.
3. SampleDetectionThreshold is the default minimum RFU for analyzing a peak on any channel. Peaks whose height lies above the detection threshold but below the minimum sample RFU (or analysis threshold) can receive artifact notices but will not be called as alleles. This value can be overridden for specific channels using the key word DetectionThresholdOverride.
4. MinInterlocusRFU is the minimum RFU for a sample peak that lies between two loci, and not falling in either locus’ extended range.
5. MinLadderInterlocusRFU is the minimum RFU for a ladder peak that lies between two loci.
6. RawDataString is either R (for raw) or A (for analyzed). This should virtually always be R.

Note: The marker set name, such as “Identifiler” and the lane standard name, such as ABI-LIZ-LESS250and340-500, are selected in the user interface using a drop-down menu, thus guaranteeing a compatible spelling. A list of available marker sets and associated lane standards is forthcoming.

One way to automatically generate this file, either completely or substantially, is to run an analysis on Windows or on a Macintosh in which the files/folders have been preconfigured to match what is intended on Linux and the OP has been preconfigured using the Osiris GUI. The correct text input file, as described above, is included in the output of the analysis accessible using the “Details” button.