Adding Marker Sets to OSIRIS

The following instructions can be used to add new markers to OSIRIS for analysis. Multiplexed marker sets with an allelic ladder can be added with the kit definition instructions. New internal markers can be added with the internal marker instructions to allow fragment analysis and to support new kit definitions.

Contents

Creating a Kit Definition Using the Ladder Generation Program

Note that it is not necessary to create new fragment analysis kits or modify the existing fragment analysis kits to add a new internal sizing marker (ILS) definition. Fragment analysis kit definitions like [LandStandardOnly_2] have access to all new and included internal markers.

Background

A "kit definition" is the information that OSIRIS needs to analyze data from a marker set, including the sizes of loci and alleles in an allelic ladder and which standard internal size markers will be used. Differences in CE instruments may sometime require different kit definitions to account for instrument-specific differences in DNA fragment migration.

Kits are defined by the allelic ladder in OSIRIS. (Fragment analysis definitions use a different process, for which kits are already defined in OSIRIS.) Creating a kit definition may also require creation of an internal size marker standard definition (ILS), if the internal marker is not already defined in OSIRIS, and may require modification of the positive control sample list if the positive control DNA sample or any loci are not already defined in OSIRIS. A kit definition with the internal marker (ILS), the positive control, thresholds and parameters is referred to as an Operating Procedure.

For OSIRIS to size allele peaks correctly, it must be able to identify the peaks of the internal marker fragments accurately. Sometimes, small internal marker fragments may be hidden by, or confused with primer or primer-dimer artifacts. Alternatively, the CE run may not be long enough to collect large internal marker fragment data. To account for those situations, internal marker definitions may exclude some small and large size fragments from consideration. OSIRIS does not need two internal marker peaks flanking the loci to accurately size allele peaks (in contrast to analysis methods that employ local or global Southern size estimation). In the instructions below, the internal size marker is referred to as the "ILS family" and a subset of internal size fragments is referred to as the "ILS".

OSIRIS uses a base pair size range on the internal marker to search for each kit locus, called the search region. Internal marker fragment migration can be affected by the dye label and by the CE instrument configuration. If changes in the dye label or CE instrument make the internal marker fragments migrate differently in comparison to the loci, OSIRIS may need an "ILS family" internal marker specific for a different dye or CE instrument. ILS families predefined in OSIRIS may be labeled with the instrument, separation medium (like POP-7 polymer), or dye-label (like WEN or CC5). Older ILS Families were historically only for the ABI instrument with POP 4 polymer and thus may not have the instrument, medium, or dye name included in the label.

Set up the Osiris-Files folder

Set up the necessary files and folders in the Osiris-Files folder by opening OSIRIS then selecting Tools>Command line tools. This will also open a command line window which can then be closed.

Supporting files are in your OSIRIS \Osiris-Files\ConfigurationTools subdirectories. These can be found using the OSIRIS menu by selecting *Tools>Show site settings folder…*:
In the \ConfigurationTools\LadderGeneration directory
(other than StandardPositiveControlList.tab, these files must not be edited):

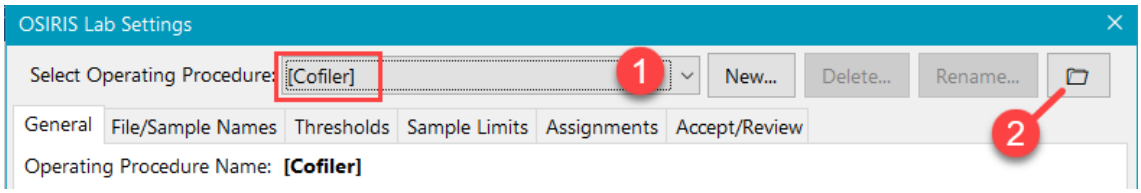
- Generic_access.txt
- Generic_LabSettings.txt
- Generic_MessageBookV4.0.xml
- Generic_StdSettings.xml
- GenericHid_LabSettings.xml

In the \ConfigurationTools\StandardPositiveControl directory:

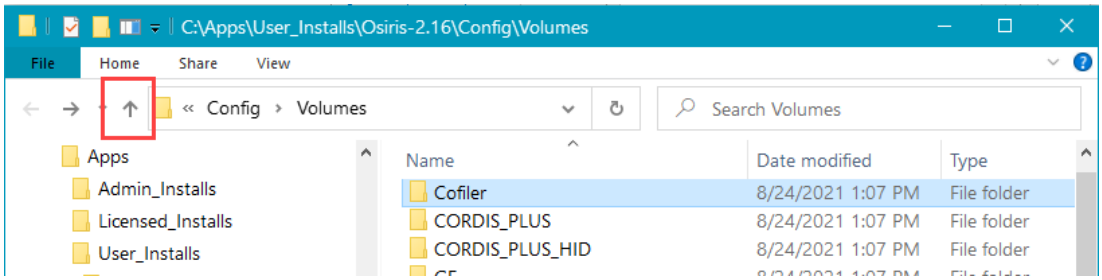
- StandardPositiveControlList.tab

Find your \LadderSpecifications directory

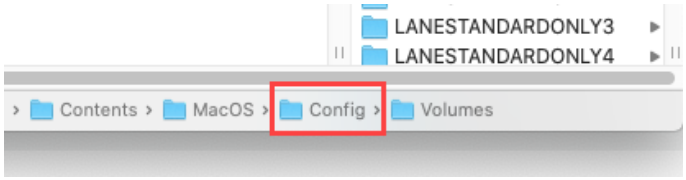
Your installation directory can be found using OSIRIS. From the OSIRIS menu, select Tools>Lab Settings>Select [Cofiler] (with brackets [ ]) from the dropdown list **(1)** >Click the folder icon **(2)** at the top right of the window. The folder window will open.



Windows:
Click the up arrow to go to the \Config folder, then select and double click the \LadderSpecifications folder, where the ILSAndLadderInfo.xml folder is located.



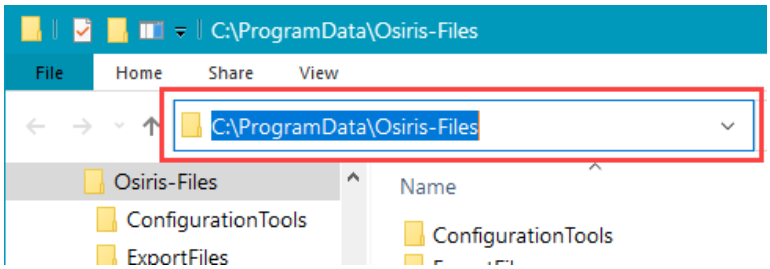
Macintosh:
Double click the “Config” folder icon at the bottom of the Finder window, then select and double click the \LadderSpecifications folder icon, where the ILSAndLadderInfo.xml folder is located.



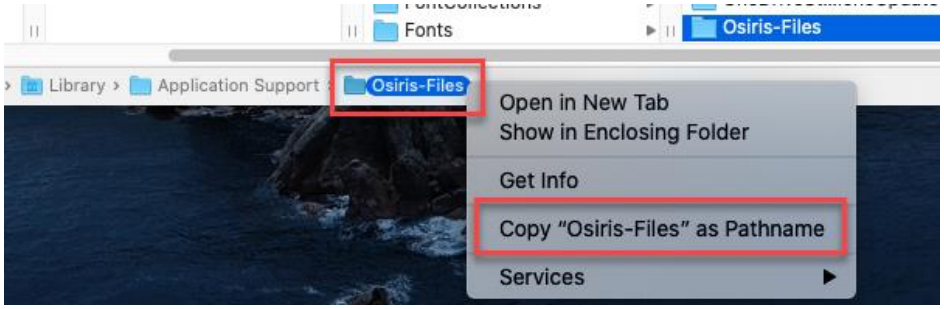
Find your \Osiris-Files folder and copy the full path name.

Your \Osiris-Files folder can be found using OSIRIS.
From the OSIRIS menu, select Tools>“Show site settings folder…”. The folder window will open.

To copy the full path name in Windows:
Click in the folder path bar to show the directory path. Copy the \Osiris-Files directory path like “C:\ProgramData\Osiris-Files”. (Your \Osiris-Files path may be different.)



To copy the full path name in the Macintosh:
Press Ctrl and click the Osiris-Files folder icon at the bottom edge of the Finder window, then select



Determine if the internal marker used with the new kit is already defined in OSIRIS. A list of predefined internal markers is in the Internal Lane Standard Markers section of Appendix L of the OSIRIS User Guide. You will need the internal marker name (ILS Family) and the name of the subset of internal marker size fragments (ILS). If your internal marker is not already defined, use the Adding a new internal marker (ILS) to OSIRIS instructions below to create it.

Verify that you have the correct names if your internal marker is already defined in OSIRIS. You will need to view the configuration file *ILSAndLadderInfo.xml* found in the \Config\LadderSpecifications folder of your installation directory with a text editor like Wordpad or Excel (PC) or TextEdit (Mac) to find the names. "ILS Family" names appear within the tags <ILSName>…</ILSName>, just beneath the tag <ILS>. The "ILS" subset names appear below the tag <LaneStandard>. Check that the sizes in your internal marker are represented in the lane standards within the named ILS family. Copy and save the ILS Family name. Including that name in the kit definition will allow the kit definitionto use any of the lane standards listed in that "ILS family".

Example:

<ILSName>ABI-ROX-500</ILSName>
<DyeName>ROX</DyeName>
<LaneStandard>
<Name>ABI-ROX500</Name>

Determine whether all of the loci and the standard positive control DNA in the new kit are already defined in OSIRIS. Open the StandardPositiveControlList.tab file in the \Osiris-Files\ConfigurationTools\StandardPositiveControl folder in a spreadsheet program and verify the following. Do not make changes to the file before you read the instructions for modifying this file. Confirm that:

- the name of your new kit's standard positive control DNA is present in the first row
- the names of all of loci in your new kit are present in the first column
- the kit's standard positive control DNA allele values for all loci in the new kit are in the kit's positive control name column

If any of those are missing, follow the Adding new Loci and Positive Controls to OSIRIS instructions below to modify the file before creating the new kit definition.

Gather required configuration files and information

Obtain these required configuration files (the names may be different, but they must be .txt files). The Bins and Panels files are generally available from the company that created the kit.

- Bins file
- Panels file

Open each of these files in a spreadsheet program to verify the format of the columns and rows. If the formats are different from the format of *PowerPlex_Fusion_Bins_CC5_tutorial.txt* and *PowerPlex_Fusion_Panels_CC5_tutorial.txt* files included in the *\Osiris-Files\ConfigurationTools\Tutorial_PPFusion* folder, you may need to perform additional steps or reformat them to match the format of those files.

If Bins and Panels files for the new kit are not available, you may be able to create them yourself following the format of the PowerPlex_Fusion bins and panels example files.

Create the kit definition

Creating a new kit definition is done in two phases. The first phase sets up the new kit's allelic ladder and internal marker. The second (optional) phase allows you to add additional internal markers (ILSFamilies).

The following instructions include both the steps to create your own kit definition (marked "a.") and also steps to create a PowerPlex Fusion kit as a tutorial demonstration (marked "b."). The tutorial below assumes you are using Windows, and that your \ConfigurationTools folder is here: C:\ProgramData\Osiris-Files\ConfigurationTools. The demonstration files in \ConfigurationTools\DemoFiles included with your OSIRIS software have been modified to reflect the full directory path to the \Osiris-Files\ConfigurationTools directory that you installed on your computer. Those "demo" files do not need to be edited.

Note that if you copy commands from this document and paste them into your input text file, be careful to remove the letter of the step ('b.') and the tab space, if they get copied. Note that you may also introduce non-printing characters which will prevent the program from running properly.

Phase 1

Creating the kit from bins and panels files:

1. Put your Bins and Panels files in a subdirectory that you create in \Osiris-Files\ConfigurationTools. (Use no spaces in the folder name).
    a. Like \Osiris-Files\ConfigurationTools*YourKitName*
    b. For the tutorial, use the bins and panels files in \Osiris-Files\ConfigurationTools\Tutorial_PPFusion

2. Create an input .txt file in in the same folder as your Bins and Panels files. This file can have any name:
    a. like *your_kit_name*_input.txt
    b. For the Tutorial create PPFusion_Tutorial_input.txt

    - All lines in input.txt file will be in the form *Command* = *value*;
    - All commands must be spelled and capitalized exactly as they appear here
    - All lines must end with a semicolon (;)

3. The first line of the input .txt file must be:
   LadderOperation = New;
   - In the following, you will modify the instruction in the 'a' steps to create your kit. Step 'b' instructions are for the PowerPlex Fusion Tutorial example.

4. The second line of the input .txt file must specify the directory path to the \Osiris-Files folder:
   a. OutputConfigPath = *directory_path_to*\Osiris-Files;
   b. OutputConfigPath = *directory_path_to*\Osiris-Files;
   - Note: all of the additional lines in the input .txt file can be in any order

5. Choose a name for the kit's allelic ladder file. It must have no spaces and end in "_LadderInfo.xml". Add this line to your input.txt file ('b.' is the Tutorial):
   a. LadderFileName = *your_ladder_name*_LadderInfo.xml;
   b. LadderFileName = PPFusion_Tutorial_LadderInfo.xml;

6. Specify the "Reagent kit" name (Spaces may be used. This is the name that will display in the OSIRIS Lab Settings window General tab beside "Reagent Kit"):
   a. KitName = reagent_kit_name;
   b. KitName = PowerPlex Fusion Tutorial;

7. Specify the full directory path to your bins and panels files in the ConfigurationTools folder, like:
   a. LadderDirectory = *directory_path_to_bins_and_panels*;
   b. LadderDirectory = *directory_path_to*\Osiris-Files\ConfigurationTools\Tutorial_PPFusion;

8. Specify the name of the "bins" file:
   a. BinsFileName = *bins_file_name*;
   b. BinsFileName = PowerPlex_Fusion_Bins_CC5_tutorial.txt;

9. Specify the name of the "panels" file:
   a. PanelsFileName = *panels_file_name*;
   b. PanelsFileName = PowerPlex_Fusion_Panels_CC5_tutorial.txt;

10. Specify the number of dyes in the kit:
    a. NumberOfDyes = *number_of_dyes*;
    b. NumberOfDyes = 5;

11. Specify the dye name for each OSIRIS display channel
    The following dye names are pre-defined in OSIRIS. If your kit uses a dye that is not defined, you can simply use one of the colors in place of the dye name.
    (Blue) 5-FAM, FL, FAM, FL-6C, Blue, BLUE,
    (Green) JOE, VIC, JOE-6C, 5-HEX, Green, GREEN,
    (Yellow) NED, TMR, TMR-ET, TAMRA, TMR-6C, L552, Yellow, YELLOW,
    (Red) ROX, PET, CXR, CXR-ET, CXR-6C, TAZ, LR600, Red, RED,
    (Orange) LIZ, CC5, GRODY, WEN-6C, WEN, BTO, DY632, AF-633, Orange, ORANGE,
    (Purple) SID, TOM-6C, TOM, TET-592, Purple, PURPLE
    Use one line for each dye (# is the number of the display channel in OSIRIS):

    a. Dye# = dye_name;
    b. The tutorial's 5 dyes require 5 lines:

Dye1 = FL;
Dye2 = JOE;
Dye3 = TMR-ET;
Dye4 = CXR-ET;
Dye5 = CC5;

12. Specify the graph display color for each channel, one color per line. The 6 available colors are BLUE, GREEN, YELLOW, RED, ORANGE, PURPLE:
    a. Color# = color_name;
    b. The 5 colors require 5 lines:

Color1 = BLUE;
Color2 = GREEN;
Color3 = YELLOW;
Color4 = RED;
Color5 = ORANGE;

13. OSIRIS requires a search text string (characters) for the default Operating Procedures. No spaces are allowed. This abbreviated name is required but will not display in OSIRIS. This should be specific for each kit generated.
    Specify it with:
    a. SearchString = *search_string*;
    b. SearchString = PPFusionTutorial;

14. Specify the name for the default Operating Procedure folder (no spaces are allowed, and the name must be unique for all the Operating Procedures). This folder will store information regarding the reagent kit, ILS, and lab settings in \Osiris-Files\Config\Volumes:
    a. VolumeDirectoryName = *volume_directory_name*;
    b. VolumeDirectoryName = PPFusion_Tutorial;

15. Specify the default expected maximum number of alleles per locus, e.g., for the majority of loci in the kit. (Locus exceptions are specified below.) The maximum expected for autosomal loci is typically 2 alleles, and is 1 allele for many Y chromosome loci:
    a. MaxExpectedAllelesPerLocusDefault = *max_expected_alleles*;
    b. MaxExpectedAllelesPerLocusDefault = 2;

16. Specify any exceptions to the default maximum number of expected alleles in a locus, with one locus per line. E.g, in an autosomal STR kit, an exception would be one or more Y-chromosome loci with a single allele expected. This step is not required if all the loci in the kit have the same expected maximum number of alleles. In the example the Fusion kit has a single Y-locus, DYS391 which is expected to have a single allele. So, the input .txt file will have a single line. (Note the colon (:) between the locus and the number of alleles):
    a. MaxExpectedAlleles = locus_name : max_expected_alleles_this_locus;
    b. MaxExpectedAlleles = DYS391:1;

17. Specify whether or not the majority of loci in the kit are Y chromosome-linked (e.g., for a Y-chromosome marker kit, "YLinkedDefault = true"; for an autosomal marker kit like the tutorial, "YLinkedDefault = False"):
    a. YLinkedDefault = *true_or_false*;
    b. YLinkedDefault = false;

18. Specify any loci that are exceptions to the YLinkedDefault specified above, with one locus per line. In a Y-chromosome marker kit, exceptions would be one or more autosomal loci. In an autosomal marker kit, exceptions would be one or more Y-chromosome loci. In the tutorial, DYS391 is the only Y-chromosome locus in the majority-autosomal Fusion kit:
    a. YLinkedOverride = *locus_name*;
    b. YLinkedOverride = DYS391;

19. Specify any loci (one per line) that do not have alleles outside the ladder range or off-ladder alleles (e.g., mono- or biallelic loci such as Amelogenin, Y-indel or quality control markers, like QS1 in the step below):
    a. DoNotExtend = *locus_name*;
    b. DoNotExtend = AMEL;

20. <u>Option for kits that include internal amplification quality control loci</u> in positive controls samples. Internal PCR amplification quality loci are not evaluated in positive controls. Failure to specify those loci here may result in significantly more editing of positive control samples. Specify each locus on a separate line:
    a. QualityLocus = *locus_name*;

    For example in the Qiagen Investigator 24plex kit:

    QualityLocus = QS1;

21. Specify the OSIRIS channel number to display the ILS internal marker, generally the last display channel. (This is not necessarily the data channel in the .fsa/.hid file):
    a. ILSChannel = *channel_number*;
    b. ILSChannel = 5;

22. Specify the "ILS Family" (internal marker) name:
    For this step and for the next step below, you should have identified these names in the Preparation for creating a new kit definition section above.
    a. ILSFamilyName = *ILS_Family_name*;
    b. ILSFamilyName = PROMEGA-ILS-CC5-500-IDX;

23. Specify the ILS name (internal marker fragment subset of the ILS Family above) :
    a. ILSName = ILS_subset_name;
    b. ILSName = Promega-ILS-CC5-500-IDX;

24. Specify the standard positive control to be used. This must appear within the list of standard positive controls in the first row of the StandardPositiveControlList.txt file (described in the Preparation for creating a new kit definition section above):
    a. StdControl = standard_positive_control_name;
    b. StdControl = 2800M;

25. Specify whether the program should generate the default lab settings for only .fsa files (*false*), or one for .fsa files and one for .hid files (*true*):
    a. HID = true_or_false;
    b. HID = true;

26. **Optional**: If a ladder locus shows stutter or other low-level, spurious peaks, the user can specify that such ladder peaks are to be ignored. This may help OSIRIS in the ladder analysis. For such loci, the user should specify:
    a. RelativeHeightOverride = *locus_name*;
    For example, RelativeHeightOverride = TPOX;

Continues on next page

27. **Optional:** Current OSIRIS 6-channel kits display the Internal marker (ILS) data in channel 6, however the ILS data is actually in the ABI analyzer's .fsa/.hid file channel 5. The GenerateLadderFile program takes that into account. If the ILS data is NOT in the .fsa/.hid file channel 5, the user should specify the data channel override command to specify which channel should be exchanged with channel 5 as follows:
    a. KitDataChannelOverride:Osiris_channel_number = .fsa/.hid_channel_number;

    If the ILS data is in .fsa/.hid channel 6 instead of 5, for example:
    KitDataChannelOverride:6 = 5;
    KitDataChannelOverride:5 = 6;
    Alternatively, for a kit where the collected sample data was in channel 2, and the ILS data was in channel 4 of the .fsa/.hid file, for example:
    KitDataChannelOverride:1 = 3;
    KitDataChannelOverride:2 = 4;

Note the channels in which sample and ILS data are collected may depend on the CE analyzer set up.

28. **Optional**: You can add expected off-ladder alleles that will appear in the accepted "Allele Assignments" accepted "Off-ladder alleles" list in the lab settings of the new kit definition so that they appear in the Lab Settings Assignments tab of the default Operating Procedure (which cannot be edited). Those off-ladder alleles in the list will not be marked as off-ladder in analyzed samples. The list of accepted off-ladder alleles can be edited (added/deleted) in custom Operating Procedure copies made from the default. Add the following command for each accepted off-ladder allele:
    a. AcceptedOL:*locus* = *allele*;

    For example:
    AcceptedOL:Th01 = 8.2;

29. Add a line with only a semicolon. This tells the program there are no more inputs:
    a. ;
    b. ;

30. Review your file to ensure correct capitalization of the 'commands' and to ensure that every line ends with a semicolon (;). If you copied the commands from this document, ensure that you have deleted any step labels ('a.' or 'b.') and any space at the beginning of the line. Copying and pasting can also introduce non-printing or other characters that could cause problems. You may be able to find non-printing characters by copying the contents of your input file into a viewing tool for non-printing characters, like https://www.soscisurvey.de/tools/view-chars.php, or some similar tool on the internet.

31. Save your input file.

32. If you performed the tutorial in the 'b.' steps, you can compare your "PPFusion_Tutorial_input.txt" input file to the "PPFusion_Tutorial_input_demo.txt" file in the \DemoFiles folder. The contents should be the same.

Run the GenerateLadderFile program

1. Start OSIRIS and close the "Recently viewed files" window. From the menu select Tools>"Command line tools…".

   On Windows. This will open a command line window with a current working directory of \Osiris-Files\ConfigurationTools.

   On Macintosh: This will open a command line window with a current working directory of ~/Library/Application Support/Osiris-Files/ConfigurationTools.

   If the following message appears:
   Warning – OK to run "/Users/*USERNAME*/Library/Preferences/.osiris/oscmdline.sh"?
   Click "Ok".

   Note that in the Mac the directory slashes are right slashes, like /Osiris-Files/ConfigurationTools

2. Change directories to the subdirectory containing your input text file, like \Osiris-Files\ConfigurationTools\YourKitName. Type the following and press enter:
   a. cd *YourKitName*
   b. For the tutorial:
      cd Tutorial_PPFusion

3. Type the following then press Enter:
   a. GenerateLadderFile < *your_kit_name*_input.txt
   b. GenerateLadderFile < PPFusion_Tutorial_input.txt

4. Copy the program output and save it to a file in case you need to troubleshoot problems.

   To copy in Windows: Use the mouse to Highlight the command line window output, press Enter to copy it to the clipboard, paste it into a document and save it. (You can also Right-click the command line window top title bar and select 'edit', then select Copy to copy highlighted Command window output.)

   To copy on the Macintosh: highlight the Command line window output with the mouse and press <command> v, to copy.

5. If the program ends with an error, check to make sure all lines in your input file end with a semicolon then check the file's command(s) mentioned in the error message. If a command is spelled or capitalized incorrectly, the error will specify the line that failed. Compare the incorrect line in your file to the instructions above. You can find non-printing characters as described above in the step about reviewing your input text file.

6. Close and restart OSIRIS. Check that your new kit appears in the Lab Settings "Select Operating Procedure" dropdown list of default kits in square brackets like [My New Kit].

7. Test the created ladder and default Operating Procedure by analyzing 5 - 10 different ladders. If necessary, save a backup copy of the *your_ladder_name*_LadderInfo.xml file, make corrections and rerun the program. Rerunning the program with your corrected input file will overwrite your original *your_ladder_name*_LadderInfo.xml file. Note that if you have created custom Operating Procedures (OP) using the newly created Operating Procedure, rerunning the program with your corrected input file will not update the custom OPs.

This is all that is required for Phase 1.

The PowerPlex Fusion kit generated in the tutorial 'b.' steps above should now work to analyze the PowerPlex Fusion data in the OSIRIS demonstration data set in the \Osiris\TestAnalysis\PowerPlexFusionHID directory.

Troublshooting:
You can test the program if you are having problems by running it with the demo file.

1. Change directories in the command line window to the subdirectory containing demo files \Osiris-Files\ConfigurationTools\DemoFiles. Type the following and press enter:
    a. cd DemoFiles

2. Type the following, then press Enter:
    a. GenerateLadderFile < PPFusion_Tutorial_input_demo.txt

Phase 2

Adding a different internal marker (ILS family) with associated search regions to the new kit definition.

If you only need to use the internal marker added in Phase 1, you do not need Phase 2.

Do not perform Phase 2 to add a new ILS to the fragment analysis Operating Procedures like [LaneStandardOnly_2].

If you are using different internal size markers with the new kit, you can add them to the new kit definition in Phase 2. This is like Phase 1, but with fewer commands in the input .txt file. Phase 2 adds information to the file generated in Phase 1. Perform Phase 2 each time you want to add an additional internal marker to your kit.

Because different dyes and CE analyzer configurations can affect the migration of internal marker DNA fragments, you may need to add a second copy of an internal size marker. An example is the Promega Internal Lane Standard 500, which requires different "ILS Family" versions for the CC5 and WEN dye-labels.

Note: Adding a new ILS to a new kit requires a new Bins file specific to the new kit with the new ILS.

If changes in the dye label or CE instrument (such as different Polymer or capillary length) make the internal marker fragments migrate differently compared to the migration of the fragments in the kit's loci, OSIRIS may need an "ILS family" internal marker specific for the different dye or CE setup so that it has the correct "search region". (See the description in the introductory Background section above.)

Before adding a new internal marker to the kit In Phase 2, determine if the internal marker you want to add to the new kit is already defined in OSIRIS. If not, create a new internal marker definition as described in the Preparation for creating a new kit definition section above.

Before performing Phase 2, make a backup copy of the *your_ladder_name*_LadderInfo.xml you created in Phase 1 so that you will not have to redo Phase 1 if you need to modify your Phase 2 input file to make corrections. An Operating procedure modified with errors in the Phase 2 input file cannot be corrected by rerunning with a corrected Phase 2 input file.

Note that the 'b.' steps are for the tutorial, as in Phase 1 above.

1. Put the Bins file for the new ILS in the subdirectory that you created for the Bins and Panels files in \Osiris-Files\ConfigurationTools during Phase 1. (Use no spaces in the folder name):
    a. Like \Osiris-Files\ConfigurationTools*YourKitName*
    b. For the Tutorial, the Bins file PowerPlex_Fusion_Bins_WEN_tutorial.txt is in the \Osiris-Files\ConfigurationTools\Tutorial_PPFusion

2. Create a new text-format input .txt file in the same location as the input .txt file in the first step of Phase 1:
    - Like *your_kit_name_add_ILS*_input.txt

    in \Osiris-Files\ConfigurationTools*YourKitName*
    - For the tutorial create

    PPFusion_Add_WEN_ILS_input.txt
    in \Osiris-Files\ConfigurationTools\Tutorial_PPFusion

3. The first line must be:
    a. LadderOperation = Amend;
    b. LadderOperation = Amend;

4. The second line of the input .txt file must specify the directory path to the \Osiris-Files folder:
    a. OutputConfigPath = *directory_path_to*\Osiris-Files;
    b. OutputConfigPath = *directory_path_to*\Osiris-Files;

5. Specify the name of the ladder file that you are adding to. This must be <u>exactly</u> the same name you used in the Phase 1 input .txt file, but <u>ending in ".xml"</u>:
    a. LadderFileName = *ladder_name*_LadderInfo.xml;
    b. LadderFileName = PPFusion_Tutorial_LadderInfo.xml;

6. Specify the full directory path and the new Bins file name for the new internal marker. This file will have different search regions than the Bins file for the first internal marker in Phase 1.:
    a. BinsFileName = *directory_path_to**bins_file_name.txt*;
    b. For Tutorial (with no return before the end of the line):

BinsFileName = *directory_path_to*\Osiris-Files\ConfigurationTools\Tutorial_PPFusion\PowerPlex_Fusion_Bins_WEN_tutorial.txt;

7. Specify the new internal marker (ILS Family) name. This <u>must</u> be different from the internal marker name in Phase 1:
    a. ILSFamilyName = *ILS_Family_name* ;
    b. ILSFamilyName = PROMEGA-ILS-WEN-500;

8. Add a line with a semi-colon:
    a. ;
    b. ;

9. Review your file to ensure correct capitalization of the 'commands' and to ensure that every line ends with a semicolon (;).

10. Save the file.

11. If you performed the tutorial in the 'b.' steps, you can compare your "PPFusion_Add_WEN_ILS_input.txt" input file to the "PPFusion_Add_WEN_ILS_input_demo.txt" file in the \DemoFiles folder. The contents should be the same.

Rerun the GenerateLadderFile program

Refer to Run the GenerateLadderFile program in Phase 1 for details in running these steps.

1. From the menu select Tools>"Command line tools…" to open a command line window.

2. Change directories to the subdirectory containing your input text file, like \Osiris-Files\ConfigurationTools\YourKitName. Type the following and press enter:
    a. cd *YourKitName*
    a. For the tutorial:
    cd Tutorial_PPFusion

3. In the command line window, type:
    a. GenerateLadderFile< your_kit_name_add_ILS_input.txt
    b. For the Tutorial:
    GenerateLadderFile < PPFusion_Add_WEN_ILS_input.txt

4. If the program ends with an error, check that all lines in your input file end with a semicolon, then check the command mentioned in the error message.

That is all that is required for Phase 2.

Phase 2 above can be repeated for each new ILS Family internal marker supported by the kit. For example, PowerPlex Fusion supports an additional internal marker: pop7-WEN-V2, specific for use with POP7 polymer.

Note: If you make a mistake in the Phase 2 input file, you cannot correct *your_ladder_name*_LadderInfo.xml by rerunning Phase 2 with a corrected Phase 2 input file. You must start Phase 2 with the backup copy of *your_ladder_name*_LadderInfo.xml.

Bins and panels file format

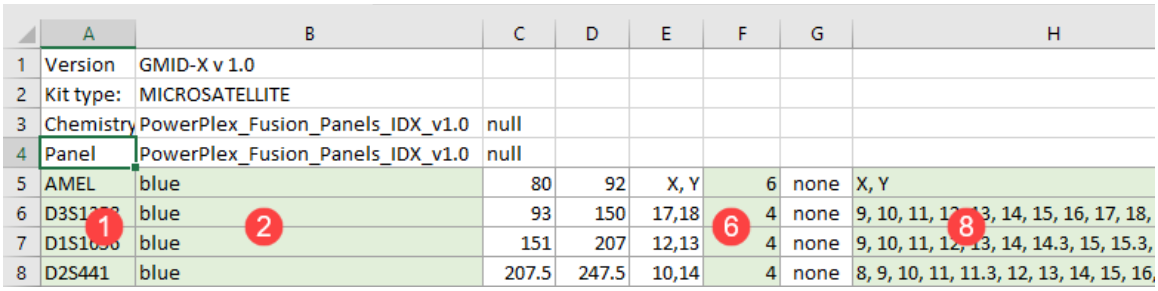
The Ladder Generation program expects the Bins and Panel files to have a certain format. If your format is different, you can:

- tell the ladder generation program the new format
- reformat the files

Expected Panels file format:

- The file is expected to be a tab-delimited text file (txt)
- The first 4 lines are information about the kit and chemistry (these are ignored).
- Line 5 starts the data used by the GenerateLadderFile.exe program, one locus per line. The data required by OSIRIS is expected in these columns:
- Column 1: Locus Name
- Column 2: Channel Color
- Column 6: repeat base pair size (Repeat Number)
- Column 8: Allele List (comma separated)
- The other columns are ignored.

Open the PowerPlex_Fusion_Panels_CC5_tutorial.txt file in the \Osiris-Files\ConfigurationTools\Tutorial_PPFusion folder using Excel or some other spreadsheet file for an example of this format:



	A	B	C	D	E	F	G	H
1	Version	GMID-X v 1.0						
2	Kit type:	MICROSATELLITE						
3	Chemistry	PowerPlex_Fusion_Panels_IDX_v1.0	null					
4	Panel	PowerPlex_Fusion_Panels_IDX_v1.0	null					
5	AMEL	blue	80	92	X, Y	6	none	X, Y
6	D3S1358	blue	93	150	17,18	4	none	9, 10, 11, 12, 13, 14, 15, 16, 17, 18,
7	D1S1656	blue	151	207	12,13	4	none	9, 10, 11, 12, 13, 14, 14.3, 15, 15.3,
8	D2S441	blue	207.5	247.5	10,14	4	none	8, 9, 10, 11, 11.3, 12, 13, 14, 15, 16,

Commands for a different Panels file format

To avoid reformatting the Panels file, include the following commands on lines in your input .txt file before the last line that has only a semicolon (;). Note: The locus names must be in the first column.

- For comma separated Panels files (.csv):

ColumnDelineation = panels_file_column_separator;

Example: ColumnDelineation = ‘,’;

- If the list of ladder alleles in the panels file is not comma-separated (like in a .csv file), the user must specify:

AlleleListDelineation = panels-file_allele_list_separator;

Example for space separated alleles: AlleleListDelineation = ‘ ‘;

- NumberOfPanelsLinesSkipped = number_of_lines_to_skip_at_panels_file_start;
- ColorColumn = column_number_for_color_in_panels_file;
- RepeatSizeColumn = column_number_for_repeat_size;
- AlleleListColumn = column_number_for_allele_list;

For example, if there were 5 lines before the data and the Colors, Repeat size and Allele list were in columns 5, 7 and 9:

NumberOfPanelsLinesSkipped = 3;
ColorColumn = 3;
RepeatSizeColumn = 7;
AlleleListColumn = 9;

Expected Bins file format:

- The file is expected to be a tab-separated text file (.txt)
- The first 4 lines are information about the kit and chemistry. These are ignored. The program will find the data lines below it.
- Line 5 starts the data used by the GenerateLadderFile.exe program.
  Each set of marker lines Starts with "Marker Name" in column 1 and *name_of_marker* in column 2.
  Following the marker name line, the data is:
- Column 1: allele
- Column 2: approximate ladder allele size in internal marker (ILS) base pairs
- Column 3: left side of allele bin, distance from the value in column 2 (base pair). (Column must be present, but values are not used by OSIRIS.)
- Column 4: right side of allele bin, distance from the value in column 2 (base pair) . (Column must be present, but values are not used by OSIRIS.)
- Column 5: whether the allele is a peak in the ladder (blank), or the allele peak is not included in the ladder ("virtual"). This is so that expected common alleles will not be marked "off-ladder" even if the ladder does not include that allele peak. Because the ladder generation program uses the first and last locus allele peaks to identify the ladder locus size range for each locus, it is important that any alleles before the first locus allele peak and after the last locus allele peak be marked as virtual.

In the example below on line 6, Amelogenin X is approximately 83.86 internal marker (ILS) base pairs with columns 3 and 4 specifying an allele bin of +/- 0.5 base pairs. While OSIRIS requires this column format, it does not use the values in columns 3 and 4. The bin width is specified in the lab settings. The first and last non-"virtual" size values for each locus in column are used for finding the ladder locus allele peaks. (E.g., Line 10, 93.91is used to find the first peak in D3S1358, because the 8 allele in line 9 is "virtual".)

	A	B	C	D	E
1	Version	GMID-X v 1.0			
2	Chemistry Kit	PowerPlex_Fusion_Panels_IDX_v1.0			
3	BinSet Name	PowerPlex_Fusion_Bins_IDX_v1.0			
4	Panel Name	PowerPlex_Fusion_Panels_IDX_v1.0			
5	Marker Name	AMEL			
6	X	83.86	0.5	0.5	
7	Y	89.61	0.5	0.5	
8	Marker Name	D3S1358			
9	8	93.91	0.5	0.5	virtual
10	9	98.05	0.5	0.5	
11	10	102.16	0.5	0.5	
12	11	106.3	0.5	0.5	

1 2 3 4 5

Command for a comma separated Bins file format
The lines and columns must be as above. Use the following command if the Bins file is comma-separated (csv), rather than a tab-separated.

- For comma separated Bins files (.csv):

BinsDelineation = bins_file_column_separator;
Example: BinsDelineation = ',';

Adding a new Internal Marker (ILS) to OSIRIS

There is no need to add or modify kit definitions for fragment analysis, all that is necessary is to add the new internal sizing marker ILS. The fragment analysis kits like [LandStandardOnly_2] have access to all newly added and pre-defined internal markers.

Background

In the instructions below, the internal size marker is referred to as the "ILS family" and a subset of internal size fragments is referred to as the "ILS". Internal marker definitions containing a subset of the total number of fragments may exclude some small and large size fragments from consideration. This allows analysis of data where small marker fragments comigrate with primer and primer dimer peaks or where the CE analysis has not been long enough to collect all of the large fragment data. The program can be configured to omit any number of small or large fragments, in case PCR primer peaks interfere with small marker peaks or small or large primer peak data is not collected in the analysis.

Preparation for creating an internal size marker definition

You will need the following:

- The name of the internal size marker
- The base pair sizes of all of the marker fragments
- The dye label name (like LIZ or WEN)
- The number of small or large fragments that you wish OSIRIS to ignore
- Optionally, the relative peak heights (if the internal marker has defined peak height differences)
- A text editor that can edit tab-separated .txt files

Create the size marker input file

1. Create an \ILSGeneration folder in \Osiris-Files\ConfigurationTools. See Find your \Osiris-Files folder.

2. Create an input .txt file in the \ILSGeneration folder like:
    a. *your_internal_marker_name*_input.txt. (No spaces in the name.)
    b. for the Internal Marker Tutorial, create the text file: Promega_ILS_CC5_500_Tutorial_input.txt
    - All lines in the internal marker input.txt will be in the form *Command* = *value*;
    - All commands must be spelled and capitalized exactly as they appear here
    - All lines must end with a semicolon (;)
    - The lines can appear in any order. All the commands are required except for the optional commands: RelativeHeightList, CorrelationAcceptance1 and CorrelationAcceptance2.

3. Specify a name for the internal marker file. The program will add "_ILSInfo.xml" to this name when generating the xml file. This name must have no spaces.
    a. ILSFileName = *your_internal_marker_name*;
    b. ILSFileName = Promega_ILS_CC5_500_Tutorial;

4. Give the full directory path to the users \Osiris_Files folder.
    a. UserFiles = *C:**ProgramData*\Osiris-Files;
    b. UserFiles = *C:**ProgramData*\Osiris-Files;

5. Give the internal marker standard name. This should be the manufacturer's name for the size marker. The name must contain no spaces.
    a. ILSFamilyName = *your_internal_marker_name*;
    b. ILSFamilyName = Promega-ILS-CC5-500-Tutorial;

6. List all of the fragment sizes in the internal marker in numerical order, separated by a space.
    a. PeakList = *list_of_peak_sizes_separated_with_a_space*;
    b. PeakList = 60 65 80 **100** 120 140 160 180 **200** 225 250 275 **300** 325 350 375 **400** 425 450 475 **500**;
    - The numbers in the tutorial are bolded to make it easier to see the differences of the numbers between the hundreds.

7. Specify the name of the dye to be used by the ILS.
    a. DyeName = *dye_name_for_ILS_channel*;
    b. DyeName = CC5;

It can be helpful to use an internal marker subset that ignores one or more small fragments in the internal marker. For example, a 40 or 60 base pair fragment can be hidden by PCR primer artifacts, or the data may not have been collected by the analyzer in that fragment size range. Similarly, if the analysis is run for too short a time, the larger internal marker fragments may not have been collected. Internal marker fragment subsets can be generated, ignoring up to *NumInitialPeaksIgnored* initial, or *NumInitialPeaksIgnored* final peak fragments. Note that theGenerateILSFamily.exe program will create all the <u>combinations</u> of ignored peaks. So if 0, 1, 2 and 3 initial fragments are ignored and 0, 1, 2, and 3 final fragments are ignored, there will be 16 different internal marker fragment subset combinations (4x4) which makes dropdown lists of available ILS subsets long. If not specified, the default for the number of peaks ignored is 3. Use 0 if you do not want to ignore peaks.

The tutorial creates the full Promega_ILS_CC5_500 internal marker and subsets that ignore the 60 and 65 base pair fragments: 60-500, 65-500, and 80-500 fragments.

8. Specify the number of initial fragments to ignore.
    a. NumInitialPeaksIgnored = *maximum_number_of_initial_sizes_to_ignore*.
    b. NumInitialPeaksIgnored = 2;

9. Specify the number of final fragments to ignore.
    a. NumFinalPeaksIgnored = *maximum_number_of final_sizes_to_ignore*;
    b. NumFinalPeaksIgnored = 0;
10. Specify a display name for the internal marker. This can be the same as the ILSFamilyName. The program will add the range of included peak sizes to this name.
    - The display name will appear in "Internal Lane Standard" list in the OSIRIS Lab Settings for all the "LaneStandardOnly" fragment analysis kits and those kits that include it when they are created.
    - For example, in the Tutorial, if you choose to ignore the one 60 base pair fragment and use "Promega-ILS-CC5-500-Tutorial_" as your BaseLaneStandardName, the program will create two markers, the full marker and the subset with one fragment ignored:
      Promega-ILS-CC5-500-Tutorial_60-500
      Promega-ILS-CC5-500-Tutorial_65-500
      a. BaseLaneStandardName = *internal_marker_display_name*;
      b. BaseLaneStandardName = Promega-ILS-CC5-500-Tutorial_;

11. **Optional:** Specify the relative heights of the lane standard peaks. If the peak heights of the internal marker fragments are reproducibly different, you can specify the approximate proportional heights of each fragment to help OSIRIS identify the correct peaks. The values are as follows.

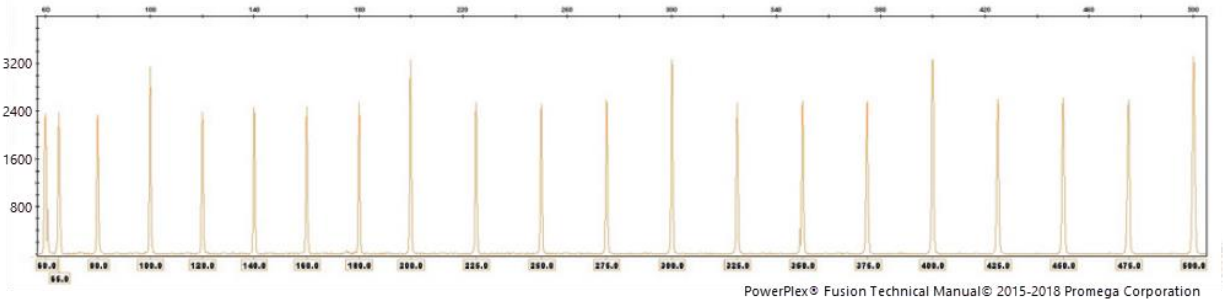
Value	Height relative to tallest peak	Proportion of tallest peak	Relative to 1000 RFU
L	low	0 – 0.2	0 – 200 RFU
ML	medium low	0.2 – 0.4	200 – 400 RFU
M	medium	0.4 – 0.6	400 – 600 RFU
MH	medium high	0.6 – 0.8	600 – 800 RFU
H	high	0.8 – 1.0	800 – 1000 RFU

OSIRIS prioritizes these specified heights in distinguishing the true ILS peaks from artifact peaks. If the true internal marker is not found on the first try, a try is attempted with these relative height constraints relaxed. If the true internal marker is not found on the second try, a third try is attempted with the relative peak heights ignored.
If specified, the values must be given for each fragment and in the same order as the fragment sizes in PeakList:

a) RelativeHeightList = *list_of_relative_heights_space_separated*;
b) RelativeHeightList = MH MH MH H MH MH MH MH H MH MH MH H MH MH MH H MH MH MH H;

See figure below:

This shows the peak heights of the tutorial internal marker, Promega ILS CC5 500:



12. **Optional**: When analyzing the internal marker (ILS) of a sample or ladder, OSIRIS selects the set of peaks that most closely matches the spacing of the fragments in PeakList above. OSIRIS may find more than one set of peaks whose spacing might represent the actual internal marker peaks. For accuracy, OSIRIS uses two correlation coefficient values to eliminate sets with poor spacing. CorrelationAcceptance1 tests the correlation with the linear spacing of the ILS sizes. The default value is 0.993.
    - Do not specify values for CorrelationAcceptance1 and CorrelationAcceptance2 unless testing shows that OSIRIS fails to analyze sample internal markers that have minimal or no artifact peaks. If OSIRIS fails to analyze clean internal markers, lower the values of CorrelationAcceptance1 and CorrelationAcceptance2 (below) by 0.2 – 0.3, then rerun the ILS generation program and reanalyze the samples. Choose the largest values for these two settings that produce reliable ILS analyses.

    a. CorrelationAcceptance1 = correlation_override_for_first_pass_acceptance;
    b. CorrelationAcceptance1 = 0.990;

13. **Optional**: CorrelationAcceptance2 tests the correlation with a non-linear transformation of the spacing of the fragments in PeakList, because the true relation of ILS peaks is known to be non-linear. This correlation is expected to be higher than the linear correlation (CorrelationAcceptance1). The default value is 0.9975.
    a. CorrelationAcceptance2 = correlation_override_for_final_acceptance;
    b. CorrelationAcceptance2 = 0.995;

14. **Important**. The last line must be a semicolon:
    a. ;
    b. ;

15. Review your file to ensure correct capitalization of the 'commands' and to ensure that every line ends with a semicolon (;). If you copied the commands from this document, ensure that you have deleted any step labels ('a.' or 'b.') and any space at the beginning of the line. Copying and pasting can also introduce non-printing or other characters that could cause problems. You may be able to find non-printing characters by copying the contents of your input file into a viewing tool for non-printing characters, like https://www.soscisurvey.de/tools/view-chars.php, or some similar tool on the internet.

16. Save your input file.

17. If you performed the tutorial in the 'b.' steps, you can compare your "Promega_ILS_CC5_500_Tutorial_input.txt" input file to the "Promega_ILS_CC5_500_Tutorial_input_demo.txt" file in the \DemoFiles folder. The contents should be the same.

Run the GenerateILSFamily program

1. Start OSIRIS and close the "Recently viewed files" window. Select Tools>"Command line tools…" from the menu.

   On Windows. This will open a command line window with a current working directory of \Osiris-Files\ConfigurationTools.

   On Macintosh: This will open a command line window with a current working directory of ~/Library/Application Support/Osiris-Files/ConfigurationTools.
   If the following appears: Warning – OK to run "/Users/*USERNAME*/Library/Preferences/.osiris/oscmdline.sh"?, click "Ok".

   Note that in the Mac the directory slashes are right slashes, like /Osiris-Files/ConfigurationTools

2. Change directories to the subdirectory containing your input text file, like \Osiris-Files\ConfigurationTools\ILSGeneration. Type the following and press enter:
   a. cd ILSGeneration
   b. For the tutorial:
      cd ILSGeneration

3. Type the following command, including the '<' and the name of your input .txt file:
   a. GenerateILSFamily < *your_internal_marker_name*_input.txt
   b. For the Tutorial:
      GenerateILSFamily < Promega_ILS_CC5_Tutorial_input_demo.txt

4. Press Enter. Copy the program output and save it to a file in case you need to troubleshoot problems:

   Windows: Highlight the command line window output with the mouse, press Enter to copy it to the clipboard, paste it into a document and save it. (You can also Right-click the command line window top title bar and select 'edit', then copy to copy highlighted Command window output.)

   Macintosh: highlight the Command line window output with the mouse and press <command>-c, to copy.

5. If the program ends with an error, check to make sure all lines in your input file end with a semicolon, including the last line, which must be just a semicolon. Then check the file's command(s) mentioned in the error message. If a command is spelled or capitalized incorrectly, the error will specify the line that failed. Compare the incorrect line in your file to the instructions above. You can find non-printing characters as described above in the step about reviewing your input text file.

6. If the program ran successfully, close and restart OSIRIS. Check that your new internal marker (ILS) appears in the Analysis window "Internal Lane Standard" dropdown list when the [LaneStandardOnly_2] kit is selected.

7. Test the created ILS with an Operating Procedure that uses it, or by using it with a fragment analysis Operating Procedure.

Adding new Locus definitions and Positive Controls to OSIRIS

Background

OSIRIS has a list of all the loci and alleles for the standard positive controls that are provided with commercially available STR analysis kits. This list allows OSIRIS to automatically check the positive controls for accuracy.

The following instructions will allow you to:

- add new loci, or define alleles for an existing standard positive control
- add a new positive control DNA with its loci/alleles

Note: New loci or positive controls must added before you create new ILS or Kit definitions above.

Preparation

Before you modify the StandardPositiveControlList.tab file, make a backup copy with a different name.

You can find StandardPositiveControlList.tab located in the /Osiris-Files/ConfigurationTools/StandardPositiveControl folder using OSIRIS: Open OSIRIS and select Tools>"Show site settings folder" from the menu.

Use a spreadsheet program (like Excel or similar program) to open the StandardPositiveControlList.tab file.
Windows: When opening the file, you may have to select the file type "All Files (*.*)" to be able to select the file in your spreadsheet program.

Mac: Use the Finder to search for the StandardPositiveControlList.tab file. Control-click the filename and select Open with>Other then select "All applications" from the Enable dropdown to open with Excel.

To use Google sheets: you need to upload the file to Google Drive, edit, then download the file, rename it to the original StandardPositiveControlList.tab name, and copy it back into the /Osiris-Files/ConfigurationTools/StandardPositiveControl folder.

The positive control DNAs and their alleles are listed in columns (like 9947A in column B) and the loci are listed in rows (like D3S1358 in row 2). Positive control DNAs may not have alleles defined at every locus. Note that amelogenin (AMEL) alleles are listed in this table as 1 (X) and 2 (Y).
It may look like this:

	A	B	C	D	E	F	G	H	I	J
1	Locus	9947A	9948	K562	DNA007	2800M	3657	MK1	M308	
2	D3S1358	14,15	15,17	16	15,16	17,18		15,17		
3	D16S539	11,12	11	11,12	9,10	9,13		12,13		
4	AMEL	1	1,2	1	1,2	1,2	1,2	1,2		
5	TH01	8,9.3	6,9.3	9.3	7,9.3	6,9.3		6,9.3		
6	TPOX	8	8,9	8,9	8	11		8,9		
7	CSF1PO	10,12	10,11,12	9,10	11,12	12		9,11		

- Determine whether your standard positive control is in the list.
- Determine whether all of you control DNA's loci are listed. NOTE: the loci are not in order.
- If you need to add to the list, save a backup copy of the original file. The file in which you make changes must be saved with the original name, StandardPositiveControlList.tab.

Add information to the StandardPositiveControlList

Note: Do not insert blank rows or columns in the data.

To add new alleles to an existing locus in a positive control

- Find the locus row and add the allele values in the correct positive control column. If there are two or more alleles, separate them with a comma, e.g, "10, 12". Enter a single allele value for a homozygote, e.g., "12", not "12, 12".

To add new loci to an existing positive control

- Review the locus list carefully to ensure that the locus is not already listed. The list is not in order. Do not add duplicate loci.
- Add each new locus to a blank line at the bottom of column A. The order of the loci is not important.
- Add the comma-separated positive control DNA alleles for the new locus in the correct column for your control DNA.

To add a new positive control DNA

- Add the new Standard Positive Control name in the first row at the first blank cell to the right (e.g., column J).
- Add comma-separated alleles for all relevant loci. For amelogenin (AMEL), use '1' for the X allele and '2' for the Y allele.
- Add new loci at the bottom of the first column. Do not add duplicate loci.

Processing the updated file

You must process the file you have modified to create an updated StandardPositiveControls.xml file in your \Osiris-Files directory.

1. Save the updated StandardPositiveControlList.tab file (with the original file name) in its original directory or it will cause errors. (The file must be saved as a tab delimited **.tab** file.)

2. Open a command line window (Windows) or a terminal application (Mac) using OSIRIS: open OSIRIS and select Tools>"Command line tools…" from the menu. The command line window will open in the \Osiris-Files\ ConfigurationTools directory.

3. Type the following in the command line window. Include the double quotes around the path. (Note there is no '<' here).

   Windows:
   BuildStandardControlFile "%OSIRISFILES%"

   Macintosh:
   BuildStandardControlFile "$OSIRISFILES"

4. Press Enter. If there are no errors, the program will list the names of the standard positive controls followed by a list of loci and associated alleles. A new StandardPositiveControls.xml file will be created in ~/Osiris_Files/Config/LadderSpecifications. That file will be used for subsequent OSIRIS analyses.

5. Test your new positive control definition. You must add Loci or Positive controls before you create your new kit or ILS definition. Once you have created your new kit or ILS:
   - Test a Positive control – create a directory that includes positive control .fsa/.hid files, and at least one sample .fsa/.hid file that has been renamed as a positive control to test positive control Quality Control. Analyze the test directory. The sample that is renamed as a positive control should give errors for the loci that do not match the positive control alleles.
   - Test an ILS – do a fragment analysis run using the new ILS or use the new ILS in the new kit to which you have added it.

Questions or comments? Email forensics@ncbi.nlm.nih.gov.