**Adding a New Kit to Osiris**

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# Adding a new kit to OSIRIS

The easiest way to add a new kit to OSIRIS is to use an existing kit as a template and modify it to apply to the new kit. If you add a new kit definition to OSIRIS for a commercially available kit and would be willing to share that with the community or if you need assistance or have questions regarding adding a new kit, please contact us: [forensics@ncbi.nlm.nih.gov](mailto:forensics@ncbi.nlm.nih.gov?subject=New%20kit%20in%20Osiris).

1. Find the files that must be modified: kitcolors.xml and ILSAndLadderInfo.xml (to locate them, go to the Osiris directory; these files are in ..\Osiris\Config\LadderSpecifications)

### New Internal Lane Standards (ILS’s)

* 1. ILSAndLadderInfo.xml:
     1. If needed, add the specifications for any new Internal Lane Standards (ILS’s)
     2. ILS names must be unique
     3. The characteristics are the sizes of the ILS peaks, each separated by a space
     4. Relative peak heights are High (H), Medium High (MH), Medium (M), Medium Low (ML) and Low (L) – in the absence of expected relative height information, use either H or MH for all.
     5. The Correlation Acceptance Threshold dictates the minimum correlation of spacing between located peaks and ideal characteristics and 0.993 has worked well (that is, accepts common spacing and rejects incorrect collections of peaks) for both ABI and Promega ILS’s
     6. Correlation Auto Acceptance Threshold dictates the minimum correlation of spacing between (quadratically) transformed located peaks and ideal characteristics (accounting for non-linear distribution of characteristics): 0.999 works well (see above) for Promega and 0.9975 works well for ABI (both with and without the “250” peak)
     7. Under the “kits” tag, near the bottom of the file, the new kit name must be specified, along with the file name for the ladder specification data. For example, for Identifiler and IdentifilerPlus:

<Set>

<KitName>Identifiler</KitName>

<FileName>Identifiler\_LadderInfo.xml</FileName>

</Set>

<Set>

<KitName>IdentifilerPlus</KitName>

<FileName>IdentifilerPlus\_LadderInfo.xml</FileName>

</Set>

### Kit Colors

* 1. kitcolors.xml:
     1. The new kit must be added, led by the kit name
     2. Each channel must be specified with colors for the Osiris Analyzed data (analyzed), the raw data (raw) and the associated ladder (ladder). The precedent is that color shades are increasingly lighter from Analyzed data>Raw data>Allelic ladder data.
     3. Generally, color data may be copied from already existing kit(s)

### Ladder

1. The Ladder Data File
   1. The file name is the same as specified in 1.a.vii (above) and the path should be the same as the kitcolors.xml and ILSAndLadderInfo.xml files
   2. Under the tag “LS”, list all available internal lane standard names and specify the channel number of the ILS. For example:

<Set>

<Name>IdentifilerPlus</Name>

<NChannels>5</NChannels>

<LS>

<LSName>ABI-LIZ500</LSName>

<LSName>ABI-LIZ450</LSName>

<LSName>ABI-LIZ400</LSName>

<LSName>ABI-LIZ350</LSName>

<ChannelNo>5</ChannelNo>

</LS>

…

* 1. File name suffix, genotype suffix and directory search string are no longer used, but must be included; any values may be used
  2. Optionally, a channel map, relating the channel in the .fsa file to the “reported” channel, can be specified…see the XML schema and the example below:

<DirectorySearchString>PP12</DirectorySearchString>

<FsaChannelMap>

<Channel>

<KitChannelNumber>1</KitChannelNumber>

<fsaChannelNumber>1</fsaChannelNumber>

</Channel>

<Channel>

<KitChannelNumber>2</KitChannelNumber>

<fsaChannelNumber>3</fsaChannelNumber>

</Channel>

<Channel>

<KitChannelNumber>3</KitChannelNumber>

<fsaChannelNumber>4</fsaChannelNumber>

</Channel>

</FsaChannelMap>

* 1. For each locus, enter:
     1. Locus name
     2. Channel number
     3. MinBP: the allelic ladder base pair that gives the low end of the range of the extended locus (may be set to be equal or less than the allelic ladder base pair of the first ladder allele).
     4. MaxBP: the allelic ladder base pair that gives the high end of the range of the extended locus (may be set to be equal or greater than the allelic ladder base pair of the last ladder allele).
     5. MinBP and MaxBP can be set to overlap neighboring loci, either core or extended, but may not exceed either neighboring locus
     6. The lab settings parameter labeled “Ignore artifacts lower than…” must be set low enough that it does not impinge on any ladder or extended alleles. If not specified in the lab settings file, this parameter defaults to ILS-ref value of the left-most ILS peak.
     7. MinGridLSBasePair: left end of the search interval for locus ladder alleles (in ladder files), specified in ILS-ref units
     8. MaxGridLSBasePair: right end of the search interval for locus ladder alleles (in ladder files), specified in ILS-ref units
     9. The two parameters above delineate the range in which the ladder locus alleles can be found and should be set conservatively large (for example, 20 – 30 ILS-ref units beyond approximate ends of ladder locus); adjacent search intervals can overlap; the values can be integer or decimal
     10. List of ladder alleles
         1. Allele name (repeat number, except X and Y for AMEL, which are designated by “1” and “2”)
         2. Curve number (unused but must be present)
         3. BP: allelic ladder, or sequence, base pairs
         4. Relative Height: as for the ILS, above; should always be specified, even if always “H” or “MH”

### Operating Procedure

1. The Operating Procedure: Standard Settings, Lab Settings, and MessageBook
   1. Standard settings:
      1. Copy an existing standard settings file
      2. Modify standard positive control(s) to reflect new kit, where BioID is the allele base pair size; for example:

<StdMarkerSetSpecifications>

<StdMarkerSetCollection>

<MarkerSetName>Identifiler</MarkerSetName>

<PositiveControls>

<PositiveControl>

<Name>9947A</Name>

<Loci>

<Locus>

<Name>D8S1179</Name>

<Allele>

<Name>13</Name>

<BioID>147</BioID>

</Allele>

</Locus>

<Locus>

<Name>D21S11</Name>

<Allele>

<Name>30</Name>

<BioID>210</BioID>

</Allele>

</Locus>

<Locus>

<Name>D7S820</Name>

<Allele>

<Name>10</Name>

<BioID>273</BioID>

</Allele>

<Allele>

<Name>11</Name>

<BioID>277</BioID>

</Allele>

</Locus>

…

* 1. Lab Settings
     1. Copy an existing lab settings file
     2. Modify any locus specific settings
     3. Modify (or remove) any positive control(s)
  2. MessageBook
     1. Copy an existing MessageBook file
     2. Modify, as needed – see documentation for MessageBook
  3. Rename all files to incorporate new kit name and place in folder with appropriate kit name (no spaces); add folder to collection of such folders in ..\Osiris\Config\Volumes

# Constructing a homebrew allelic ladder

Osiris requires an allelic ladder to perform sample analysis. Therefore, if using a custom made STR multiplex, the user will have to construct an allelic ladder to serve as a control sample. Typically allelic ladders are made by pooling individual samples to give a good representation and distribution of alleles and allele repeat sizes. Given that Osiris uses a cubic spline to estimate allele sizes from the allelic ladder, each locus should have at least four to five alleles at a minimum, with as wide an allele repeat range as possible. More alleles and a wider range will increase the accuracy of allele calling. Three or fewer alleles might not work well. The ladder allele peak heights do not need to be similar to one another and having gaps where an allele is not present in the ladder is not a problem. In fact, the ladder fitting algorithm is designed such that different peak height and allele gaps in the ladder actually increase the robustness of ladder fitting. Osiris does not require that ladder alleles flank all of the identified alleles for allele calling. If the sample alleles fall outside the ladder allele range, Osiris will extrapolate to call the alleles. In our somewhat limited experience of Osiris extrapolation (using commercially available kits), Osiris performance calling alleles outside the ladder range by extrapolation was excellent.

To construct an allelic ladder de novo, select samples that have the alleles of interest and pool the DNA. Amplify, analyze the pool, rebalance the amounts of the inputs to the pool to achieve the desired peak balance and reamplify the rebalanced pool to make the ladder. Once a satisfactory ladder has been achieved, the amplified ladder can be stored as a frozen stock for amplifying new batches of allelic ladder for analysis. Reamplification of ladder can be done by making a 1:1000 dilution of the amplified ladder stock and amplifying 5 ul for 15 rather than 28 cycles (to avoid over-amplification of ladder peaks). Serial amplification of ladders should be avoided to prevent degradation of ladder characteristics or introduction of artifacts.