

IX.A.25. Off-ladder allele policy: DISCARD/RARE

X. Osiris enhancements for DNA·VIEW IMPORT/EXPORT

X.A. Import/Export – import DNA profile data

X.A.4. Genotyper import file format

X.A.7. File format details

X.A.7.e. Comment column (optional)

The optional comment column is for a comment that applies to the case as a whole. (Any comments specific to a single sample should be put at the end of the Sample Info cell, as a “name or comment”.)

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X.A.8. DNA·VIEW and Osiris

(from version 33.07 – 2014)

Profile data can be exported from Osiris and imported into DNA·VIEW.

Add an export option to Osiris as explained below which will create data files including RFU values acceptable to DNA·VIEW for import.

The DNA·VIEW "Genotyper" file format (§X.A.4) can include, in addition to allele sizes, also rfu (and other) information.

X.A.8.a. What to export from Osiris

X.A.8.a.i.) Export anything that might be an allele

For DNA·VIEW mixture calculation, data should be exported from Osiris with minimal filtering. A low detection threshold, about 35rfu, is desirable. Signals that look like stutter should be exported allowing DNA·VIEW to make decisions.

X.A.8.a.ii.) But do use Osiris' clever artifact diagnostics

- » I think it is proper to allow Osiris to filter out (not export) signals for which Osiris base-pair estimate is not close to an integer, as representing trash rather than true alleles. Osiris uses the word "residual" to mean the error between estimated base pair size and the nearest integer.
- » Similarly, Osiris' parameter $\text{Fit} < 0.98$ (possibly even $\text{Fit} < 0.99$ from limited experience) is probably diagnostic of illegitimate data.

Osiris version 2.3 seems to allow automatic filtering based on a residual threshold, but require manual editing to utilize the Fit data.

X.A.8.b. Osiris "Lab settings" configuration

Create customized Osiris lab settings operating procedures (in Osiris: Tools ► Lab settings) by modifying standard Osiris operating procedures (NEW... ► **Select an operating procedure to copy**).

The following screen shots show my parameter choices. Note that the choice **fsa** vs. **hid** is part of the operating procedure (in the **General** tab). To switch between these formats I manually edit the operating procedure.

OSIRIS Lab Settings

Select Operating Procedure: [CHB Identifier] New... Delete... Rename...

General | File/Sample Names | Locus/ILS Thresholds | **Sample Thresholds** | Assignments | Accept/Review

RFU Limits

	Sample	Ladder	ILS
Analysis Threshold (RFU)	35	200	200
Min. Interlocus RFU			
Max. RFU			
Detection Threshold (RFU)			

☒ Allow User to Override Min. RFU

Sample Limits

	Value	Units
Max. No. of pullups peaks per sample	2	peaks
Max. No. of stutter peaks per sample		peaks
Max. No. of adenylation peaks per sample	1	peaks
Max. off-ladder alleles per sample:	1	peaks
Max. residual for allele (<0.5 bp):	0.1	Sample/Ladder BP alignment
Max. No. of peaks with excessive residual:		peaks
Incomplete profile threshold for Reamp More/Reamp Less:	5000	RFU
Ignore artifacts smaller than:	25	bps
Total Number of Samples With Excessive Pullup		
Percent of Samples With Excessive Pullup		%
Maximum Number of Tri-Allelic Loci in Unmixed Sample		
Maximum Number of Homozygous Loci		
Maximum Number of Triallelic Loci		
Maximum Percentage of Peaks with Pullup		%
Maximum Percentage of Alleles With Excessive Residuals		%
Maximum Number of Craters in Sample	0	
Maximum Number of Spikes in a Sample		
Summary Locus: Maximum Number of Sample Loci with Craters		
Summary Locus...Percent of Loci With Craters		%
Raised Baseline Threshold for Samples (RFU)		
Raised Baseline Threshold for Sample ILS Channels (RFU)	250	
Minimum Height for Primer Dimer Peaks (RFU)	2000	
Minimum Number of Peaks per Channel in Primer Dimer for Negative Control	2	
Percentage Of Standard Noise Threshold For Peak Identification (Default = 100)		

< Back Next > Lock OK Cancel Apply

Sample Thresholds

	Value
Test for Presence of Sub-Analytic Peaks in Negative Controls	☒
Test for Excessive Noise Above Analysis Threshold (checked) or below (unchecked)	☒
Enable Test for Excessive Noise	☒
Make Pull-up At Allele Artifact Non-Critical	☐
Flag Mixed Samples And Triallelic Loci	☐
Recommend Amp More On Low Homozygote	☐
Select Reamp Regular (checked) Versus Reinject (unchecked)	☒
Recommend Rework if Laser Off Scale Found	☒
Attempt to Apply Embedded Color Correction Matrix	☐
Ignore noise analysis in smoothing when above detection threshold	☒
Test for Primer Dimer Peaks in Negative Controls	☒
Apply Fractional Filter To Peaks Below Analysis Threshold (Homozygous Loci)	☐
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 Report Heterozygous Imbalance If Sample Is Mixture	☒
Test Adjusted Signal Heights Relative To Baseline (Overridden by below)	☒
Normalize Raw Data Relative To Baseline (Overrides above)	☒
Ignore Effects of Negative Relative Baseline	☒

< Back Next > Lock OK Cancel Apply

Locus Limits for Samples

	Default
Channel	
Fractional filter (0 - 1.0)	0
Pullup fractional filter (0 - 1.0)	0
Max. stutter threshold (0 - 1.0)	0
Max. plus stutter threshold (0 - 1.0)	
Adenylation threshold (0 - 1.0)	0.333
Min. heterozygote balance (0 - 1.0)	0
Min. homozygote threshold (RFU)	10

Min. homozygote threshold units: RFU

X.A.8.c. Osiris export for DNA·VIEW

X.A.8.c.i.) **DNAVIEW\util\RFUetcTable.xsl** is a custom DNA·VIEW export template for Osiris as discussed in Appendix E of the Osiris documentation.

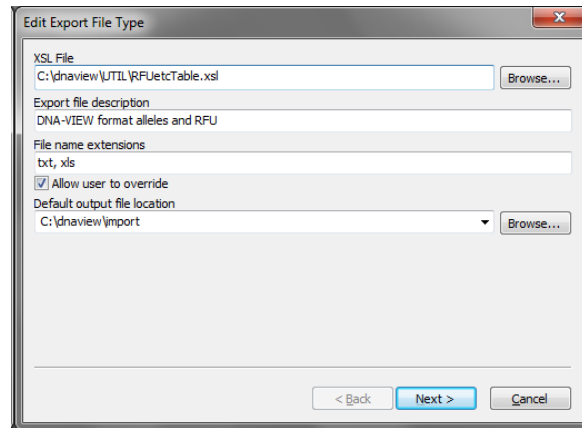
X.A.8.c.ii.) Osiris "Export file type" configuration

The Osiris manual, "Export Setup Tutorial" explains the procedure. Briefly:

In Osiris, **Tools** ▶ **Export file settings** ▶ **NEW...** and follow the screen shot at right.

The remaining screens should fill correctly

automatically. Now you have a new export menu item in Osiris: **File** ▶ **Export** ▶ **DNA·VIEW format alleles and RFU ...**

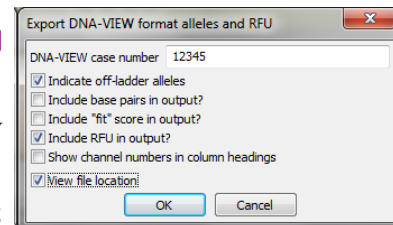


X.A.8.d. Exporting data – preparation in Osiris

X.A.8.d.i.) Open the *.oer file, review the data and delete (uncheck) noise such as bad Fit that won't be automatically filtered.

X.A.8.d.ii.) **File** ▶ **Export** ▶ **DNA·VIEW format alleles and RFU ...**

- » Check **RFU** to provide DNA·VIEW with intensity information.
- » The **base pairs** and **"fit"** boxes are for future development; DNA·VIEW doesn't consider that data at present.
- » The case number – whatever you type which can include DNA·VIEW *Case number formatting* if you use it – will be included in the output file.
- » Role letters aren't included in the output so you have to add them manually; hence the check mark in **View file location**.



X.A.8.d.iii.) 8Manually editing the output file

Open the exported text file in a text editor or (safest) in Excel.

- » Wherever you see (*role?*), substitute a suitable role letter. Don't bother for controls or ladders which are of no interest to DNA·VIEW and are not part of a case. (It is unnecessary though harmless to delete uninteresting lines.)
- » Add any comment including language and race codes for the case in the first (**Comment**) column (per §X.A.7.e). Be sure not to add or delete any tab characters. (That's the reason Excel is safest).

X.A.8.e. Import into DNA·VIEW

X.A.8.e.i.) Choose whatever treatment of off-ladder alleles is appropriate (§IX.A.25) – probably **Treat as a rare allele**.

X.A.8.e.ii.) **Import/Export** ▶ *Genotype import*