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STRmixTM Probabilistic Genotyping Software Operating Instructions

This procedure describes the use of STRmix[™] V2.4 for the interpretation of PowerPlex® Fusion DNA profiles within the NYC Department of Forensic Biology. Readers are also referred to the STRmix[™] v.2.4 Users and Operation manuals for additional information.

I. Preparing Data for a STRmixTM Analysis

- A. Before performing your STRmix[™] analysis, the following actions must be taken:
 - 1. Verify that the sample is suitable for STRmixTM analysis (Refer to the STR Results Interpretation manual)
 - 2. Determine the best described Number of Contributors to the sample (NOC). Refer to the STR Results Interpretation manual regarding the procedure for determining the number of contributors.
 - 3. Create folders for the STRmixTM runs:
 - a. Navigate to the M:\STR_Data\STRmix Data Folder
 - b. Within the STRmix Data folder, create a new folder with the FB (or FBS) case number

e.g. FB16-01234 or FBS16-05678

c. Within the FB (or FBS) case file folder, create a folder for <u>EACH</u> evidence or suspect sample that will be run through STRmixTM. Use the sample's OCME ID for the naming of the folder. Suffixes such as 'mcon' or 'reamp' should not be included.

<u>e.g.</u> FB16-01234_567_1_1.1_trig_GS FB16-01234_890_1_1.1_shirt_BL FB16-01234_123_1_1.1_VS_SF FBS16-05678_999_1_1.1_(s)JS FBS16-05678_888_1_1.1_cupJS

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Note: If a suspect is being compared to multiple FB's, create a sub-folder within that suspect sample folder for each cross-referenced FB.

- 4. Confirm that the STR data is prepared correctly for STR mix^{TM} analysis:
 - a. Evidence samples must be edited to remove all artifacts, including pull ups, spikes, dye blobs, n-8 stutter and n-2 stutter, etc before inputting into STRmixTM. Standard forward and reverse stutters must *not* be removed before importing into STRmixTM
 - b. Evidence samples must only be amplified in PowerPlex Fusion[®] in order to undergo STRmixTM analysis and an attempt should be made to amplify conditioned and comparison samples in PowerPlex Fusion[®] as well. If being utilized, comparison and conditioned samples must be edited to remove all artifacts and all stutter.
 - c. STRmixTM allows the user to calculate a likelihood ratio when the evidence and conditioned /comparison samples are analyzed in different autosomal typing kits. LRs will only be provided for those loci in common between the two kits.
 - i. In the case of an evidence and conditioned/comparison sample amplified using different autosomal kits, both the evidence and the conditioned/comparison samples have to input from .txt files and the locus order must match that of the evidence (PowerPlex Fusion[®] order). The comparison/conditioned sample data can be converted to the proper PowerPlex Fusion[®] order and appropriate .txt file format using the following macros:

For suspect, victim, elimination samples run in Identifiler use macro: **"Identifiler to Fusion Exemplar STRmix Input Creation".**

For deconvoluted DNA Donors, use macro: "Fusion Deconvoluted STRmix Input Creation"

Non numeric values such as OL or OB, < or >, and R are not permitted within the STRmixTM input files. Unambiguous alleles including those that are rare should appear in the corresponding input file as their actual allelic size designation, for example D21: 30.1

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- i. Assignment of an actual allelic size designation to a non numeric value within the input file:
- a) Open the STRmix .txt file associated with the appropriate STR project (e.g. in Notepad)
- b) Locate the sample and locus containing the non numeric value within the .txt file and manually replace the value with the appropriate actual allelic size designation
- c) Save .txt file replacing the original file
- e. Locations that contain a tri-allelic pattern or have an unresolved peak must be ignored for proper STRmixTM analysis.
 - i. Ignoring a tri-allele locus in a single source evidence sample during STRmixTM deconvolution:
 - a) In **Configure Analysis** window click **Run Settings** at the bottom of the window.
 - b) Under **Thresholds** settings click **Ignore Loci** button.
 - c) Select the locus within the **Include** window and click the > button to move the locus to the **Exclude** window.
 - d) Click save when all appropriate loci have been added to the **Exclude** window.
 - e) Click save again in the **Run Settings** window and continue with instructions for performing STRmixTM deconvolution below in section **II.**
 - ii. Ignoring a locus with unresolved peaks during LR from previous analysis:
 - a) In **Configure Analysis** window click **Run Settings** at the bottom of the window.
 - b) Under **Thresholds** settings click **Ignore Loci** button.

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c) Se lo	lect the locus within the Include window and click cus to the Exclude window.	t the > button to move the
d) Cl	ick save when all appropriate loci have been added	to the Exclude window.
e) Cl pe	ick save again in the Run Settings window and control of the set	ntinue with instructions for on III.
f. Sample da standard in "Exporting manual. g. Prepare th	ta must be assembled into the appropriate format for put for STRmix TM are .txt files . See "STRmix TM g Exemplar Table for STRmix TM input" sections wi	or STRmix [™] input. The analysis for Evidence" and thin the GeneMarker
g. Trepare un		
i. Are yo Decor	ou performing a deconvolution on an evidence samp volutions in STRmix TM	ole? Go to Section II:
ii. Are yo sample Likeli	bu performing STRmix TM to evaluate a comparison e and generate an LR, with or without conditioned p hood Ratio calculations with STRmix TM .	sample against an evidence profiles? Go to Section III:
 II. Deconvolutions in ST A. Launch the STRr task bar or by doub 	TRMIXTM. nixTM application. Open the STRmix TM software bole clicking on the STRmix TM icon on the desktop.	y locating STRmix TM in the

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B. Select "Start Analysis" from the startup screen. This will open the "STRmix – Configure Analysis" window.

🗧 STRmix - Configure Ana	lysis
STRmix: Case details Case Number Sample ID Case Notes	
Step 1: MCMC settings	1
DNA kit used	OCME_Fusion
# MCMC accepts	500000
# burnin accepts	100000
STRmix V2.4.05 - User: kmck	Run Settings Cancel Confirm

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C. Naming STRmixTM runs

STRmixTM output folder and file names are created by stringing together the values entered into the "Case Number" and "Sample ID" fields in the software followed by the date and time of the analysis run. The information in the file name is separated by dashes. Therefore, if other characters are entered, such as a comma, underscore, period, etc., the software will convert them into dashes.

Use the following naming convention for deconvolutions:

Case Number = YY-XXXXX (leave out "FB") Sample ID = remainder of the OCME ID* #p (NOC) cond elim initials (if applicable) Case Notes = leave blank

*Suffixes such as 'mcon' or 'reamp' should not be included in the OCME ID.

e.g. deconvolution of 3 person mixture, FB16-01234_567_1_1.1_trig_GS, conditioned on John Doe

Case Number = 16-01234 **Sample ID** = 567_1_1.1_trig_GS 3p condJD

Examples	Resulting STRmix file name
1-person deconvolution	16-01234-567-1-1-1-trig-GS-1p
2-person deconvolution, no conditioning	16-01234-567-1-1-1-trig-GS-2p
2-person deconvolution, conditioning victim AB	16-01234-567-1-1-1-trig-GS-2p-condAB
3-person deconvolution, no conditioning	16-01234-567-1-1-1-trig-GS-3p
3-person deconvolution, conditioning elim CD	16-01234-567-1-1-1-trig-GS-3p-condCD
3-person deconvolution, conditioning elims CD and EF	16-01234-567-1-1-1-trig-GS-3p-condCD EF

D. Set the Number of Contributors.

 Ensure that the following "Step 1: MCMC settings" are in place: DNA kit used: OCME_Fusion # MCMC accepts: 500000
 # burnin accepts: 100000

NOTE: the # MCMC accepts and # burnin accepts must not be modified without documented approval from the technical leader.

Select "run settings" to confirm run settings. The settings should be as follows for every STRmixTM analysis. Any changes that are made will appear in bold on the run report. Press "Cancel" when done. See section I.A.4.e above for instructions on ignoring loci under specific situations.

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Variance		Thresholds	
9.1374,0.7472	Allelic Variance	50,50,50,50,50,50,50 Detection threshold Edit	
1.5007,12.9748	Stutter Variance	8000 Saturation	
0.0065	Locus Amp Variance	0.3 Stutter max	
0.5	Var > mode	0.1 Forward stutter max	
Degradation		Ignore Loci	
0.01 Degr	adation max	мсмс	
-1.0 Degr	adation starts at (bp)	8 Number of chains	
Drop-in		Low Memory Mode	
100.0 Drop	-in cap	Extended Output	
0.0024 Drop-in frequency 9.0 Post burn-in shortlist			
0.0,0.0 Drop-in gamma parameters 0.005 Random Walk SD			
		Seed	
Random 948367			
Cancel Save			

- 3. Select "Confirm" to proceed to the "Add Profile Data" window, or cancel to return to the Startup screen (canceling will not save any data up to this point). Once "Confirm" is hit, a folder will be created in the STRmix results folder on your C drive. If incorrect nomenclature was used and you return to this screen to make changes, the empty folder on your STRmix results drive should be deleted.
- E. Add your Evidence Profile Data.
 - In the "STRmix Add Profile Data" window, select "Add Profile". This will bring you to the "Adding Evidence Profile Data" window (see step 2 below) where you can select either a text file or STRmix file from which to run a STRmixTM analysis. Alternatively, you can navigate to your data folder and drag and drop the appropriate text file into the top box, and proceed to step 4.

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🖉 STRmix - Add Profile Data	×
Step 2: Add Evidence Profile Data	
Add Profile	
Remove Profile	
Add Reference Profile Data	
	Contributor to: Hp Hd
Add Profile	
Remove Profile	
Change Hd	
Cane	el Back Confirm settings
STRmix V2.4.05 - User: kmckay	

- 2. In the "Adding Evidence Profile Data" window (see below), ensure that "Alleles per locus" is set to 15.
- 3. Choose "Select Text File" to navigate to and import your STRmix STR run data from the STR data folder.

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Adding Evidence Pr	rofile Data	×		
Step 2: Add Evidence	Step 2: Add Evidence Profile Data			
Profiling Kit in use: OCME_Fusion				
Import from plate	e text file:	Select Text File		
Chosen File:				
Chosen sample(s):	Chosen sample(s) from file	Alleles per locus: 15		
Export filename(s):	Name(s) to save sample(s) as	Edit		
Choose previous S Chosen File:	STRmix Evidence file:	Select STRmix File		
STRmix V2.4.05 - User:	kmckay	Add Profile Data		

4. When you select a text file with multiple samples, it will look as follows:

ſ	🗏 Choose Profile Sampl	
	- Choose Profile(s) 01-Allelic_Ladder_1_A01_001.f 04-M1_C4_100_1-4_6M_22F_L 05-M2_C2_500_2-1_15M_5F_E 05-M2_C2_500_1-1_15M_5F_F	
	4 111	
	Cancel Add	

5. Select the appropriate sample by clicking on it, then select "Add" for the relevant text file. Your view will appear as follows:

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Adding Evidence P	rofile Data	X
Step 2: Add Evidence	Profile Data	
Profiling Kit in use:	OCME_Fusion	
Import from plate	text file:	Select Text F
Chosen File: Newto	n041916 11 Experiment 8.txt	
Chosen sample(s):	06-M2_C2_500_1-1_15M_5F_F01_011.fsa	Alleles per locu 15
Export filename(s):	06-M2_C2_500_1-1_15M_5F_F01_011.fsa.	Edit
Choose previous S Chosen File:	TRmix Evidence file:	Select STRm
STRmix V2.4.05 - User:	kmckay	Add Profile Data

6. Now select "Add Profile Data" and your text file name will appear in the "Add profile Data" screen as seen below. **Repeat steps 1-6 to add any replicates of the sample which were amplified.**



F. <u>Deconvolutions without a conditioned contributor</u>. Select "Confirm settings" and this will open up the "Population Settings" window. For a deconvolution without a conditioned contributor, population data is not needed, therefore the populations will appear grayed out in the screen below. Select "Start" to begin your analysis. Proceed to Step H.

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₩ STRm	ix - Population Settings	X
Step 3	Population Settings	
NIST	AfAm Add Population Remove Population	
Pop	lation Proportion FST Allele Freq File	
Range		
Protil	e MLE for contributor # under Hp and Hd	
Factor	vie informed MX priors	
Sampli	Ig Variation	
HPD	erations: 1000 V Quantile: 99 V Sides: 1 V	
Save	as default Cancel Back Start Start &	Search
STRmix	/2.4.05 - User: kmCkay	

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G. <u>Deconvolutions with a conditioned contributor</u>: For deconvolutions with a conditioned contributor, you must also add reference profile data. Refer to the STR Results Interpretation Manual for guidance on when a conditioned contributor may be applied. A deconvolution of the evidence sample without conditioning and a LR against a potential conditioned contributor may need to be run first.

- 1. Select "Add profile" under the "Add Reference Profile Data" section of the screen, or drag and drop the file in to the reference sample box and skip to step 3.
- 2. Click on "Select Text F..." to navigate to the case conditioned reference text file.
- 3. Once you open up the reference text file, click on it and select "Add". This will bring you to the following "Adding Reference Profile Data" screen. Select "Add Profile Data" to complete the process of adding your conditioned contributor's profile to the analysis.
- 4. The conditioned sample will now appear in the "Add Reference Profile Data" section of the screen below.

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🗧 STRmix - Add Profile I	Data 📃
Step 2: Add Evidence Prof	file Data
Add Profile	17-M1_C1_750_1-2_6M_22F_601_013.fsa.csv
Remove Profile	
Add Reference Profile Dat	a Contributor for
	нр на
Add Profile 2 Remove Profile	20-22F_D03_007.fsa.csv X
Change Hd	
	Cancel Back Confirm settings
STRmix V2.4.05 - User: km	nckaý

5. Conditioned contributors are considered true donors in Hp and Hd. Therefore, you must assign the conditioned contributors as such by selecting "Change Hd". This will allow the conditioned contributor to be chosen under H_p and H_d and will have an "X" marked in both columns.

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STR	mix - Add Profile Data 2: Add Evidence Profile Data Add Profile emove Profile emove Profile Add Profile emove Profile Change Hd Cancel Back Confirm setti x V2.4.05 - User: kmckay	ngs

6. Select "Confirm Settings". This will open up the Populations Settings window. When a reference sample is conditioned, population data is needed in the calculation. Ensure that the following four populations are listed: NIST Caucasian, NIST Asian, NIST AfAm, and NIST Hisp and that the settings appear as below.



FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS STRmixTM Probabilistic Genotyping Software Operating Instructions Document ID: 6482 Status:Published APPROVED BY DATE EFFECTIVE PAGE 15 OF 28 01/10/2017 Nuclear DNA Technical Leader STRmix - Population Settings Х Step 3: Population Settings NIST AfAm Add Population Remove Population Population Proportion FST Allele Freq File NIST Caucasian 0.03b(1.0,1.0) NIST1036_strmix_Ca.. 0.25 NIST Asian 0.25 0.03b(1.0,1.0) NIST Fusion Asian.csv NIST AfAm 0.25 0.03b(1.0,1.0) NIST Fusion AfAm.csv NIST Hisp 0.25 0.03b(1.0,1.0) NIST Fusion Hisp.csv Range Profiles originates from 2 ... 2 contributors Use MLE for contributor # under Hp and Hd Stratify contribut Use informed Mx prior Factor N ✓ Display Factor of N! LR User informed Mx prior Sampling Variation Calculate HPD Include MCMC uncertainty HPD iterations: 1000 Quantile: 99 Sides: 1 Save as default Cancel Back Start Start & Search STRmix V2.4.05 - User: kmckay

H. After selecting "Start", the "Calculation Progress" window will open showing the Burnin progress and Main MCMC Progress.



Cancel calculation View Results

I. When the analysis is complete, the raw data report will open as follows:

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STRmix V2.4.05 - User: kmckay	A
Analysis run: 2016/10/28 10:58:31	
Sample ID: 1	
comments:	
Seed: 858107	
Considering Evidence as originating from	- 2 individual(s)
Pre-Burnin time taken: 0.085 secs	
Burnin time taken: 15.635 secs	
post Burnin time taken: 7.821 secs	
I otal iterations	
1121406.0	
Effective Sample Size	
15/18.048/48232828	
Average log(likelihood)	
61.91809147572932 Colmon Dubin convergence diagnostic	
4 040447700E021487	
Varianco	
5.5	
Stutter Variance	
1 0	
DNA Amounts	
0	
The information above will be saved to your S	TRmix Directory:
a la	
C:\ProgramData\STRmix\results\STRmix Deco	onvolution-1-2016-10-28-10-46-35
	Advanced Report v3.0.7 Run Report Finis

J. Select "Run Report" from this screen to create the advanced report PDF which will be saved in the STRmix results folder for this analysis. Choose "Create Report" from the screen below



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K. The analysis run name will auto-populate based on your Case and Sample ID naming. Hit "Save". The advanced report PDF will now open. Print the report.

L. Close out of the Advanced Report window and then select "Finish" on the "STRmix - Calculation Results" window. This will return you to the STRmix start up screen.

M. Navigate to your STRmix Run Folder within the STRmix Results folder. **MOVE your STRmix Run folder** into the previously created FB sample folder within the STRmix Data folder.

II. Likelihood Ratio calculations with STRmixTM:

Note: All samples must undergo deconvolution prior to running an LR with a comparison sample. Refer to the STR Results Interpretation Manual for guidance on when a conditioned contributor may be applied. A deconvolution of the evidence sample without conditioning followed by an LR against a potential conditioned contributor may need to be run first.

A. Launch the STRmixTM application. Open the STRmix software by locating STRmix in the task bar or by double clicking on the STRmix icon on the desktop. Select "LR from Previous Analysis".



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B. Navigate to the folder where the STRmix deconvolution on the M drive (STRmix data) for the relevant sample is saved. Double click on the "settings.ini" file for the sample to select it.

C. The STRmix - "Configure Analysis" window will open.

D. Naming STRmixTM Likelihood Ratio runs

The Case Number and Sample ID will auto-populate from the deconvolution settings file. **This must be updated to the appropriate naming convention for an LR run before proceeding.** Use the following naming convention:

- 1. Evidence Files
 - a. **Case Number** = YY-XXXXX (leave out "FB")
 - b. Sample ID = remainder of the evidence sample OCME ID*_scenario for LR
 - c. **Case notes** = leave blank

e.g. LR of 2 person mixture, FB16-01234_567_1_1.1_shirt_BL, comparing to elim John Doe Case Number = 16-01234 Sample ID = 567_1_1.1_shirt_BL JD1Uv2U

- 2. Suspect Files
 - a. **Case Number** = SYY-XXXXX (leave out "FB")
 - b. **Sample ID** = OCME ID* for evidence sample (leave out "FB")_scenario for LR
 - c. **Case Notes** = leave blank

*Suffixes such as 'mcon' or 'reamp' should not be included in the OCME ID

e.g. LR, FBS16-05678 suspect Tom Smith, comparing to 3 person mixture, FB16-

01234_567_2_1.1_slide_GS

Case Number = S16-05678

Sample ID = 16-01234_567_2_1.1_slide_GS TS2Uv3U

<u>Note</u> – naming format for the LR scenarios should start with the comparison sample's initials, followed by any conditioned samples' initials, and then the number and "U" for unknowns, followed by a "v" to separate the numerator from the denominator hypotheses

Examples	Resulting STRmix file name
Evidence File	
elimAB vs 1 unknown	16-01234-567-1-1-1-shirt-BL-ABv1U
elimAB+2 unknowns vs 3unknowns	16-01234-567-1-1-1-shirt-BL-AB2Uv3U
elimAB+cond elim CD vs cond elim CD+1 unknown	16-01234-567-1-1-1-shirt-BL-ABCDvCD1U
Suspect File	
suspTS vs 1 unknown	S16-05678-16-01234-567-2-1-1-slide-GS-TSv1U

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suspTS+1 unknown vs 2 unknowns	S16-05678-16-01234-567-2-1-1-slide-GS-TS1Uv2U
suspTS+cond elim CD vs cond elim CD+1 unknown	S16-05678-16-01234-567-2-1-1-slide-GS-TSCDvCD1U
suspTS+cond elim AB+1 unknown vs cond elim AB+2	S16-05678-16-01234-567-2-1-1-slide-GS-TSAB1UvAB2U
unknowns	

E. Select "Confirm". Once "Confirm" is hit, a folder will be created in the STRmix results folder on your C drive. If incorrect nomenclature was used and you return to this screen to make changes, the empty folder on your STRmix results drive should be deleted.

F. In the "Add Profile Data" window, this is where you will import comparison input files and set hypotheses. Select "Add Profile" and navigate to the .txt file for the comparison samples and select the file(s). Alternatively, you may drag and drop .txt files of your reference samples into the Reference Profile Data box. Click "Add Profile Data".



G. Each comparison sample (suspect, elim, or informative victim), that is added will be within the numerator of the LR (ie. assigned to H_p). If adding another comparison sample, repeat steps E and F for that comparison sample.

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H. NOTE: If you are conditioning on a contributor, that conditioned sample should already be in the numerator (Hp) and denominator (Hd) from the deconvolution. Conditioning profiles may not be added at the LR step.

I. Then select "Confirm settings".

J. The Population Settings window will open. Ensure that the following four populations are present in the list: NIST Caucasian, NIST Asian, NIST AfAm, NIST Hisp, as seen below. Ensure that the settings are as pictured in the screen shot below.

	Settings		
Step 3: Population Setti	ngs		
NIST AfAm	Add Population	Remove Population	
Population	Proportion	FST	Allele Freq File
NIST Caucasian	0.25	0.03b(1.0,1.0)	NIST1036_strmix_Ca
NIST Asian	0.25	0.03b(1.0,1.0)	NIST Fusion Asian.csv
NIST AfAm	0.25	0.03b(1.0,1.0)	NIST Fusion AfAm.csv
NIST Hisp	0.25	0.03b(1.0,1.0)	NIST Fusion Hisp.csv
Use MLE for contril	outor # under Hp an	d Hd [*] Stratify cont	ributor #
Factor N!		Use informed Mx prid	ors
V Display Factor of N	! LR	User informed N	1x priors
		[
Sampling Variation			
Sampling Variation		Include MCMC unce	rtainty
Sampling Variation	•	✓ Include MCMC unce Quantile: 99 ▼	rtainty Sides: 1 🔻

K. Select "Start" to calculate the LR.

L. The Calculation Progress screen will open and the software will progress through burnin and Main MCMC Progress.

M. The STRmix calculations Results window will then open. Choose "Run Report" ***CAUTION: If you it "Finish" a Run Report will NOT be generated, and the analysis will have to be re-done. N. The Advanced Report window will open. Select "Create Report".

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O. The STRmix PDF report will open and will save in the relevant folder and then close the report.

P. Close out of the Advanced Report window and then select "Finish" on the "STRmix - Calculation Results" window. This will return you to the STRmix start up screen.

Q. Navigate to your Run Folder in the STRmix Results folder on your C drive. **MOVE your STRmix Run folder** into the previously created FB Case folder in the STRmix Data folder.

IV. How to Run STRmixTM using Batch Mode

- A. A number of STRmix deconvolutions can be set up and queued to run sequentially. Note, if STRmix[™] cannot model the data by the chosen NOC, the batch mode will stop and <u>no data will</u> <u>be saved</u>.
- B. To set up a queued analysis for multiple samples, select Batch Mode from the STRmix main window.



C. Select "Add to Batch" from the Batch Mode window to open the Sample Summary window.

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Batch Mode			
	Samples in Batch		
Add to Bat	ch		^
Plugin Configu	ration		
Delete anal	rsis		
Start Bate	h		
Stop Bate	h Change batch directory		
Exit Batch M	ode		
C:\ProgramData	STRmix\results		
STRmix V2.4.05 - U	er: kmckay		

- D. Complete the analysis set up for the first sample following steps **II. Deconvolutions in STRmix**TM
- E. In the Population Settings window, select "Start" to return to the Batch Mode window.
- F. In the Batch Mode Window, select "Add to Batch" to enter the next sample. Repeat steps IV.C-E to add additional samples.

Note: to remove a sample from the batch mode, highlight the case/sample in the "Samples in Batch" section of the "Batch Mode" window then select "Delete analysis".

- G. Select "Start Batch" to start the batch run.
- H. After completion of analyses, select "Exit Batch Mode" to return to the STRmixTM main window.
- I. Results folders from Batch Mode will be saved in the STRmix Results folder on your C drive. Move the results folders from the C drive to the appropriate FB folders within the STRmix Data drive.

V. Evaluation of the STRmixTM Analysis

- A. Verify input files against the evidence input section of the STRmixTM printout and the electropherograms
 - 1. Correct input file(s) have been selected
 - 2. All appropriate edits were made, no artifact peaks were left labeled
 - 3. No allelic or stutter peaks have been removed

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- B. The number of contributors that best describes the data has been chosen
- C. The correct assumptions (conditioning) have been made, if applicable
- D. The appropriate proposition has been selected, if applicable
- E. Check the Parameters table against the settings listed below to ensure that the correct settings were used for the run (note that any edited values are bolded by the program).

For Interpretation (Deconvolutions)

Setting	Value	Setting	Value	Setting	Value
Allele Variance	9.1374,0.7472	Drop-in Cap	100.0	HPD Iterations	0
	mode=6.080				
Stutter Variance	1.5007,12.9748	Drop-in Frequency	0.0024	HPD Significance	0.0
	mode=6.496			Value	
Minimum	0.5	Drop-in Parameters	0.0,0.0	HPD Sides	0
allowed					
variance from					
the mode					
Loci	23	RWSD	0.005	Alleles Per Locus	15
Locus	0.0065	ESS Thinning	100000	Factor of N!	Yes
Amplification					
Variance					
Maximum	0.3	MCMC Accepts	500000	MCMC Uncertainty	Yes

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Stutter					
Forward Stutter	0.1	Maximum	0.01	Burn-in Accepts	100000
Max		Degradation			
Excluded Loci	DYS391	Saturation	8000	Chains	8

For Likelihood Ratios (Comparison)

Setting	Value	Setting	Value	Setting	Value
Allele Variance	9.1374,0.7472	Drop-in Cap	100.0	HPD Iterations	1000
	mode=6.080				
Stutter Variance	1.5007,12.9748	Drop-in Frequency	0.0024	HPD Significance	0.99
	mode=6.496			Value	
Minimum	0.5	Drop-in Parameters	0.0,0.0	HPD Sides	1
allowed variance					
from the mode					
Loci	23	RWSD	0.005	Alleles Per Locus	15
Locus	0.0065	ESS Thinning	100000	Factor of N!	Yes
Amplification					
Variance					
Maximum Stutter	0.3	MCMC Accepts	500000	MCMC Uncertainty	Yes
Forward Stutter	0.1	Maximum	0.01	Burn-in Accepts	10000
Max		Degradation			0
Excluded Loci	DYS391	Saturation	8000	Chains	8

F. Verify that the following make intuitive sense when compared to the electropherogram(s):

- 1. The mixture proportions assigned to the contributor(s)
- 2. The weights assigned to the genotypes listed in the Genotype Probability Distribution
- 3. The degradation values and Locus Efficiencies (LSAE)
- G. Evaluate the following diagnostics for the run information. Note that the presence of a single suboptimal diagnostic is not necessarily an indication that rework is required. In some instances suboptimal diagnostics may be due to poor quality data and not due to an issue with the run.
 - 1. Total number of iterations and acceptance rate. A very low acceptance rate (e.g. 1 in thousands to millions) may, in combination with the other diagnostics, indicate that the analysis needs to be run for additional iterations.
 - 2. Effective sample size (ESS). A low ESS in relation to the total number of iterations suggests that the MCMC has not moved very far with each step or has a low acceptance rate. A low

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ESS value (tens or hundreds) means that there is potential for a large difference in weights if the analysis is run again.

- **3.** Average (log) likelihood. The larger this value, the better STRmix[™] has been able to describe the observed data. A negative value suggests that STRmix[™] has not been able to describe the data very well given the information it has been provided. A low or negative value for the average log₁₀ (likelihood) may indicate to users that the analysis requires additional scrutiny.
- **4.** Gelman-Rubin diagnostic. If this value is above 1.2 then it is possible that the analysis has not converged.
- **5.** Allele variance and stutter variance. These variances should be compared to the mode. If the numbers are significantly elevated, the analysis may require additional scrutiny.
- **6.** Evaluate the **Per Locus Likelihood Ratio** table per locus and per sample, if applicable; pay special attention to outliers and/or zero values.

Observation	Action
A low or negative average (log) likelihood	Reevaluate number of contributors; consider another
	STRmix TM analysis with one higher or one lower
	number of contributors
	Data has been removed that is allelic and/or stutter,
	and must be re-imported
	Artifact peaks have been left labeled and must be
	removed
Gelman-Rubin value is greater than 1.2	Consider another STRmix TM analysis at greater
Y	number of iterations. Note: this requires approval by
	the Technical Leader
Stutter and/or allele variance significantly	Check to make sure no data has been omitted
elevated above mode (may be in conjunction	
with low average (log) likelihood)	
	Reevaluate number of contributors; consider another
	STRmix [™] analysis with one higher or one lower
	number of contributors

Troubleshooting Guide

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	Consider amplifying a replicate if one has not already	
	been done or increased input amount	
Large LR's (>1) obtained for each locus, except	Data entry problem—check input files	
one where the $LR = 0$ and the POI reference is		
consistent with the evidentiary profile		
	Allele call not fully resolved at a given locus – ignore	
	locus and perform analysis again. Note: discuss with	
	supervisor as needed	
	Inhibition has occurred—microcon to clean and	
	reamplify sample	
	Consider amplifying a replicate if one has not already	
	been done or increased input amount	
	Reevaluate number of contributors; consider another	
	STRmix TM analysis with one higher or one lower	
	number of contributors	
	Consider another STRmix TM analysis at greater	
	number of iterations Note: this requires approval by	
	the Technical Leader	
The mixture proportions do not reflect what is	Reevaluate number of contributors: consider another	
observed AND/OR the degradation does not	STRmix TM analysis with one higher or one lower	
reflect what is observed AND/OR the	number of contributors	
interpreted contributor genotypes do not make		
intuitive sense		
	Inhibition has occurred—microcon to clean and	
	reamplify sample	
	Consider another STRmix [™] analysis at greater	
	number of iterations. Note: this requires approval by	
	the Technical Leader	
An error occurred	The number of contributors selected was too few and	
An error occurred while executing the analysis - Calculation failed:	must be increased	
Pre-Burnin Determine Genotypes failed:		
Locus o in the evidence cannot be explained given the parameters you ha	ve chosen	
ОК		
<u></u>		
The STP mix TM run does not progress at the	Check "alleles per locus" setting is set to 15	
MCMC burn in stage and the chains do not	Check aneles per locus setting is set to 13.	
within our in stage and the chains do not		

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move. Error message also received that					
Degradation started at "0".					
STRmix - Calculation Progress					



References:

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STRmixTM v. 2.4 Users Manual

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