STRmix Probabilistic Genotyping Software Operating Instructions

Status: Published		Document ID: 6482
DATE EFFECTIVE	APPROVED BY	PAGE
08/26/2021	Nuclear DNA Technical Leader	1 OF 40

# STRmix<sup>TM</sup> Probabilistic Genotyping Software Operating Instructions

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

For STRMix<sup>™</sup> set-up instructions please refer to <u>QC702 in the Quality Control Procedures Manual</u>.

# **1** Preparing Data for a STRmix<sup>TM</sup> Analysis

- 1.1 Before performing your STRmix<sup>TM</sup> analysis, the following actions must be taken:
  - 1.1.1 Verify that the sample is suitable for STRmix<sup>TM</sup> analysis (Refer to the <u>STR Results</u> <u>Interpretation – PowerPlex Fusion & STRmix manual</u>)
  - 1.1.2 Determine the best described Number of Contributors to the sample (NOC). Refer to the <u>STR Results Interpretation manual</u> regarding the procedure for determining the number of contributors.
  - 1.1.3 Evaluate your replicates. If there are drastic inconsistencies with the alleles present between replicates, only the amplification with the most information should be used or a third amplification may be warranted.
  - 1.1.4 Create folders for the STRmix<sup>TM</sup> runs:
    - 1.1.4.1 Navigate to the M:\STR\_Data\STRmix Data Folder
    - 1.1.4.2 Within the STRmix Data folder, create a new folder with the FB (or FBS) case number

e.g. FB16-01234 or FBS16-05678

1.1.4.3 Within the FB (or FBS) case file folder, create a folder for EACH evidence or suspect sample that will be run through STRmix<sup>™</sup>. 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

1.1.4.4 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.

STRmix Probabilistic	Genotyping Softwa	re Operating Instructions	
	Ochoryphig Doriva	ne operating monuctions	

Status: Published		Document ID: 6482
DATE EFFECTIVE	APPROVED BY	PAGE
08/26/2021	Nuclear DNA Technical Leader	2 OF 40

- 1.1.5 Confirm that the STR data is prepared correctly for STRmix<sup>TM</sup> analysis:
  - 1.1.5.1 Evidence samples must only be amplified in PowerPlex Fusion<sup>®</sup> in order to undergo STRmix<sup>TM</sup> analysis.
  - 1.1.5.2 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 STRmix<sup>TM</sup>. Standard forward and reverse stutters must not be removed before importing into STRmix<sup>TM</sup>.
  - 1.1.5.3 Sample data must be assembled into the appropriate format for STRmix<sup>TM</sup> input. The standard input for STRmix<sup>TM</sup> are .txt files. See "STRmix<sup>TM</sup> analysis for Evidence" and "Exporting Exemplar Table for STRmix<sup>TM</sup> input" sections within the <u>GeneMarker</u> <u>manual</u>.
  - 1.1.5.4 Reference samples must be edited to remove all artifacts and all stutter. Incomplete or triallelic loci must not be imported into STRmix<sup>™</sup> for a reference sample – remove all allele(s) for that locus within the text file. If a possible drop-in peak is present in a reference sample, remove this peak from the text file before STRmix<sup>™</sup> import.
  - 1.1.5.5 An attempt should be made to amplify reference samples in PowerPlex Fusion<sup>®</sup>. If unavailable, STRmix<sup>TM</sup> allows the user to calculate a likelihood ratio when the evidence and reference samples are analyzed in different autosomal typing kits. LRs will only be calculated for those loci in common between the two kits.
  - 1.1.5.6 In the case of a reference sample not amplified in Fusion, the samples must input from .txt files and the locus order must match that of the evidence (PowerPlex Fusion® order). The reference sample data can be converted to the proper PowerPlex Fusion® order and appropriate .txt file format using the following macro: "Identifiler to Fusion Exemplar STRmix Input Creation".
  - 1.1.5.7 If a DNA donor is being used from one sample to condition or compare to another, use the following macro: "Reference profile for STRmix Input Creation"
  - 1.1.5.8 Non-numeric values such as OL or OB, < or > are not permitted within the STRmix<sup>™</sup> 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. If an actual allelic size designation cannot be determined, the data for this locus should be removed completely from the text file and the locus (or loci) should be ignored.
  - 1.1.5.9 In order to modify a STRmix<sup>TM</sup> input text file:
    - 1.1.5.9.1 Open the STRmix .txt file associated with the appropriate STR project (e.g. in Notepad)

	tatus: Publis	STRmix Pr	obabilisti	ic Genot	yping So	ftware Op	perating 1	Instructions
	DATE F	FFFCTIVE		APPR	OVFD BY	-		PAGE
	08/26/2021		Nuc	$1 \text{ or } \mathbf{DNA}$	Technical	Landar		3 OF 40
	00/2	20/2021	Inuc	hear DNA	Technica			3 01 40
	1.1	.5.9.2 Locate and ma .5.9.3 Save .tr	the sample nually repla at file replace	and locus on ace the valucing the ori	containing the with the aginal file	the non-num appropriate	eric value actual allel	within the .txt file ic size designation
			1	8	0			
	1.1.6	Evidence samp STRmix decom- field within the example, data n	les should b volution is j STRmix ar nust be igno	be evaluated performed. nalysis to ir pred at loci	d to determ A commer ndicate why that contai	ine if a locus at should be the locus w n:	s needs to b added to th vas ignored	be ignored before e "Case Notes" for that run. For
	1.1.6.1	a tri-allelic pa	ittern					
	1.1.6.2	an unresolved	l allelic or s	stutter peak	that is visi	ble above th	e AT	
	1.1.6.3	an OB/OL all	ele or stutte	er peak that	cannot be	assigned a c	orrect allel	ic designation
	1.1.6.4	a stutter or all locus B (igno	lelic peak for re both loci	or an allele	belonging	to locus A is	s being call	ed in a neighboring
	1.1.6.5	where a cond sample	itioning san	nple does n	ot have da	ta at a locus	that is pres	ent in the evidence
	1.1.6.6	The technical	leader show	uld be cons	ulted for a	ny situation	not covered	l above.
	1.1.7	Loci should not sample. Ensure described in 1.1	be ignored that your re5.4.	l for likelih eference sa	ood ratio ca mple text f	alculations d ile has been	lue to a par updated ap	tial comparison propriately as
	1.1.8	Prepare the scen	narios to be	run in STF	Rmix <sup>™</sup> .			
	1.1.8.1	Are you perfo Deconvolutio	orming a de <u>ns in STRn</u>	convolution nix.	n on an evi	dence sampl	le? Go to S	Section $\underline{2}$
	1.1.8.2	Are you comp sample in ord <u>STRmix</u> .	paring a ref er to genera	erence sam ate an LR?	ple against Go to Sec	a previously tion <u>3 Likeli</u>	y deconvolu hood Ratio	uted evidence calculations with
	1.1.8.3	Are you performance sample to ger Likelihood R	orming a de lerate the L atio Calcula	convolution R in a singlations (Con	n of an evid le step? Go nbined) in S	dence sample to Section <u>4</u> STRmix.	e and comp Deconvol	paring of a reference ution and
2	Dec	onvolutions	in STRn	nix <sup>TM</sup> .				
2.1	Laun task ł	ch the STRmix <sup>TI</sup> oar or by double	<sup>M</sup> applicatio clicking on	n. Open the the STRm	e STRmix <sup>™</sup> ix™ icon c	<sup>M</sup> software bon the deskto	by locating	STRmix <sup>TM</sup> in the

FURENSIC B	SIULUGY PRUTUCULS FUR FUREINS	IC STR ANALYSIS
STRmix Pr Status: Published	obabilistic Genotyping Software Oper	ating Instructions
DATE EFFECTIVE	APPROVED BY	PAGE
08/26/2021	Nuclear DNA Technical Leader	4 OF 40
₹ STRmix	STRMIX. RESOLVE MORE DNA MIXTURES.	

EQDENCIA DIAL

Start Analysis

LR from Previous Analysis

Search Database

STRmix V2.4.02 - User: jbright

PROTOCOL & EOD FORENCIC OTD A NAL VOIG

Settings

Model Maker

Batch Mode

Exit

About

2.2 Select "Start Analysis" from the startup screen. This will open the "STRmix - Configure Analysis" window.

ĕ STRmix - Configure Ana	ilysis
STRmix: Case details	
Case Number	
Sample ID	
Case Notes	
Step 1: MCMC settings —	
Number of contributors	1
DNA kit used	OCME_Fusion
# MCMC accepts	500000
# burnin accepts	100000
	Pup Settings Cancel Confirm
	Concer Committee
STRmix V2.4.05 - User: kmck	ay

STRmix Probabilistic	Genotyning	Software C	nerating	Instructions
STRIIIX TIUUAUIIISIIC	Ochotyping	Soliwale	perating	monucions

Status: Published		Document ID: 6482
DATE EFFECTIVE	APPROVED BY	PAGE
08/26/2021	Nuclear DNA Technical Leader	5 OF 40

#### 2.3 Naming STRmix<sup>TM</sup> runs

- 2.3.1 STRmix<sup>TM</sup> 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.
- 2.3.2 The following naming convention should be used 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** = a comment should be added here if a locus is ignored, indicating the reason: e.g. "D2S441 was ignored due to an unresolved allelic peak"

\*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

- 2.4 Set the number of contributors
- 2.5 Ensure that the following "Step 1: MCMC settings" are in place:

# DNA kit used: OCME\_Fusion # MCMC accepts: 500000 # burnin accepts: 100000

- 2.5.1 NOTE: the # MCMC accepts and # burnin accepts must not be modified without documented approval from the technical leader (or his/her designee).
- 2.6 Select "run settings" to confirm run settings. The settings should be as follows for every STRmix<sup>™</sup> analysis. Any changes that are made will appear in bold on the run report. If a locus needs to be ignored, follow the procedure below. Otherwise, press "Cancel" when done.

E EFFECT	TIVE	APPI	ROVED BY	PAGE
8/26/2021		Nuclear DNA	Technical Leader	6 OF 4
≶ STRmix ·	- Run Settings	5		
Run Settin	gs			
Variano	e		Thresholds	
9.1374,0	.7472	Allelic Variance	50,50,50,50,50,50,50 Detection thr	eshold Edit
1.5007,1	2.9748	Stutter Variance	8000 Saturation	
0.0065		Locus Amp Variance	0.3 Stutter max	
0.5		Var > mode	0.1 Forward stutter max	
Degrada	ation		Ignore Loci	
0.01	Degradation	max	мсмс	
-1.0	Degradation	starts at (bp)	8 Number of chains	
Drop-in			Low Memory Mode	
100.0	Drop-in cap		Extended Output	
0.0024	Drop-in freq	uency	9.0 Post burn-in shortlist	
0.0,0.0	Drop-in gam	nma parameters	0.005 Random Walk SD	
			Seed	
			<b>Random</b> 948367	

EADENSIC DIALACY DUATACALS EAD EADENSIC STD ANALYSIS

2.6.1 If a locus needs to be ignored for the deconvolution:

2.6.1.1 Under Thresholds settings click Ignore Loci button.

- 2.6.1.2 Select the locus within the **Include** window and click the > button to move the locus to the **Exclude** window.
- 2.6.1.3 Click save when all appropriate loci have been added to the **Exclude** window.
- 2.6.1.4 Click save again in the **Run Settings** window.

2.7 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 the M 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.

**2.8** Add your Evidence Profile Data.

STRmix Probabilistic Genotyping Software Operating Instructions			
Status: Published		Document ID: 6482	
DATE EFFECTIVE	APPROVED BY	PAGE	
08/26/2021	Nuclear DNA Technical Leader	7 OF 40	

2.8.1 In the "STRmix – Add Profile Data" window, select "Add Profile". This will bring you to the "Adding Evidence Profile Data" window (see step 2.8.2 below) where you can select either a text file or STRmix file from which to run a STRmix<sup>TM</sup> analysis. Alternatively, you can navigate to your data folder and drag and drop the appropriate text file into the top box, and then proceed to step 2.8.4.

🖉 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
Cancel Back Confirm settings

- In the "Adding Evidence Profile Data" window (see below), ensure that "Alleles per 2.8.2 locus" is set to 15.
- Choose "Select Text File" to navigate to and import your STRmix<sup>TM</sup> STR run data from the 2.8.3 STR data folder.

Adding Evidence Pr	rofile Data	×		
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	STRmix Evidence file:	Select STRmix File		
Chosen File:				
		Cancel Add Profile Data		
STRmix V2.4.05 - User:	kmckay			

STRmix Probabilistic Genotyping Software Operating Instructions			
Status: Published		Document ID: 6482	
DATE EFFECTIVE	APPROVED BY	PAGE	
08/26/2021	Nuclear DNA Technical Leader	8 OF 40	

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

MX	Choose Profile Sampl
	Choose Profile(s)
	01-Allelic_Ladder_1_A01_001.f 04-M1_C4_100_1-4_6M_22F_C
	05-M2_C2_500_2-1_15M_5F_E 06-M2_C2_500_1-1_15M_5F_F
	Cancel Add

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

Adding Evidence P	rofile Data	×	
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	TRmix Evidence file:	Select STRm	
Chosen File:			
Cancel Add Profile Data			
STRmix V2.4.05 - User:	kmckay		

STRmix Probabilistic Genotyping Software Operating Instructions

Status: Published		Document ID: 6482
DATE EFFECTIVE	APPROVED BY	PAGE
08/26/2021	Nuclear DNA Technical Leader	9 OF 40

Now select "Add Profile Data" and your text file name will appear in the "Add profile 2.8.6 Data" screen as seen below. Repeat steps 2.8.1-2.8.6 to add any replicates of the sample which were amplified.

STRmix - Add Profile Data	X
Step 2: Add Evidence Profile Data	
Add Profile 06-M2_C2_500_1-1_15M_5F_F01_03	11.fsa.csv
Remove Profile	
	Contributor to:
	Hp Hd
Add Profile	
Remove Profile	
Change Hd	
Cancel Back	Confirm settings
STRmix V2.4.05 - User: kmckay	

Deconvolutions without a conditioned contributor. Select "Confirm settings" and this will 2.9 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 2.10.6.1.

STRmix - Population	Settings			
Step 3: Population Settin	igs			
NIST AfAm 🔻	Add Population	Remove Population		
Population	Proportion	FST	Allele Freq File	
Range				
Profiles originates from	2 2 contributors			
Use MLE for contrib	utor # under Hp and	Hd Stratify contrib	utor #	
Factor N!		Use informed Mx priors		
Display Factor of N!	LR	User informed Mx	priors	
Sampling Variation				
Calculate HPD		Include MCMC uncerta	ainty	
HPD iterations: 1000 V Quantile: 99 V Sides: 1 V				
Save as default		Cancel Back S	tart Start & Search	
STRmix V2.4.05 - User: k	mckay			

STRmix Probabilistic Genotyping Software Operating Instructions

Status: Published		Document ID: 6482
DATE EFFECTIVE	APPROVED BY	PAGE
08/26/2021	Nuclear DNA Technical Leader	10 OF 40

- 2.10 **Deconvolutions with a conditioned contributor:** 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.
  - 2.10.1 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 <u>2.10.3</u>.
  - 2.10.2 Click on "Select Text F..." to navigate to the case conditioned reference text file.
  - 2.10.3 Once you open 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.
  - 2.10.4 The conditioned sample will now appear in the "Add Reference Profile Data" section of the screen below.

Step 2: Add Evidence	file Data Profile Data	<b>X</b>
Add Profile Remove Profile	07-M1_C1_750_1-2_6M_22F_G	01_013.fsa.csv
Add Reference Profile Add Profile Remove Profile	 Data ] 20-22F_D03_007.fsa.csv	Contributor to: Hp Hd X
Change Hd	Cancel Ba	ck Confirm settings
STRmix V2.4.05 - User	: kmckay	

2.10.5 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.

FORENSIC B	SIOLOGY PROTOCOLS FOR FORENSIC	J SI K ANAL Y SIS	
STRmix Pr	obabilistic Genotyping Software Opera	ting Instructions Document ID: 6482	
DATE EFFECTIVE	APPROVED BY PAGE		
08/26/2021	Nuclear DNA Technical Leader 11 OF 40		
	STRmix - Add Profile Data          Step 2: Add Evidence Profile Data         Add Profile         07-M1_C1_750_1-2_6M_22F_601_013.fsa.csv         Remove Profile         Add Reference Profile Data         Add Reference Profile Data         Add Profile         20-22F_003_007.fsa.csv         Hp         Hd         Change Hd         Cancel       Back         Confirm settings         STRmix V2.4.05 - User: kmckay		

2.10.6 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. Select "Start" to begin your analysis.

NIST_AfAm 👻	Add Population	Remove Population	
Population	Proportion	FST	Allele Freq File
NIST_AfAm	0.25	0.03b(1.0,1.0)	NIST Fusion AfAm_Ame
NIST_Asian	0.25	0.03b(1.0,1.0)	NIST Fusion Asian_Ame
NIST_Cauc	0.25	0.03b(1.0,1.0)	NIST Fusion Cauc_Amen
NIST_Hisp	0.25	0.03b(1.0,1.0)	NIST Fusion Hisp_Amen
ange	rom 2 to 2 contributors tributor # under Hp and	s Hd ☑ Stratify contributor	*
ange	rom 2 to 2 contributors tributor # under Hp and	Hd Stratify contributor	2
ange Profiles originates fr Use MLE for con actor N! V Display Factor o	om 2 to 2 contributors tributor # under Hp and fNI LR	Hd Stratify contributor	# 
ange	om 2 to 2 contributors tributor # under Hp and fNI LR	Hd Stratify contributor	# riors
ange	om 2 to 2 contributors tributor # under Hp and f NI LR	S Hd Stratify contributor Use informed Mx priors User informed Mx p	# riors retainty

Controlled versions of Department of Forensic Biology Manuals only exist in the Forensic Biology Qualtrax software. All printed versions are non-controlled copies. © NYC OFFICE OF CHIEF MEDICAL EXAMINER Qualtrax template 072220

STRmix Probabilistic Genotyping Software Operating Instructions			
Status: Published		Document ID: 6482	
DATE EFFECTIVE	APPROVED BY	PAGE	
08/26/2021	Nuclear DNA Technical Leader	12 OF 40	

2.10.6.1 Applying User Informed Mixture Proportions (Mx) Priors: \*\*THIS FUNCTION SHOULD NOT BE USED WITHOUT PRIOR APPROVAL BY THE TECHNICAL LEADER\*\*

User Informed Mixture Proportions (Mx Priors) is a function within STRmix that allows users to set approximate mixture proportion percentages for each contributor. If a proposed genotype does not fit the proportion percentage set by the user within the set variance, a penalty will be applied to this iteration similarly to an overall poor fit to the observed profile.

- 2.10.6.1.1 This function is limited to use with two or three person mixtures with extreme mixture proportions (ex. 98%:2% or 97%:2%:1%) with at least some of the minor contributor(s) peaks being labeled.
- 2.10.6.1.2 Using the stutter filtered electropherograms, determine an approximate mixture proportion percentage for each contributor. This should be done by calculating mixture proportions percentages, based off labeled peak heights in the first few loci of each dye channel and averaging them together. If no minor peaks are labeled at a location, use the mixture percentage proportion of 100%:0% for that locus in a two-person mixture and 100%:0%:0% in a three-person mixture in the average. If five peaks are seen at a location for a two-person mixture, do not use that location in the average.
- 2.10.6.1.3 Once the Population Settings Window is open, check the box marked "User informed Mx priors". This will open a new window like the one seen below:



2.10.6.1.4 Ensure contributor 1 is chosen in the drop down menu. Using the top slider bar marked "mean", set the mean to the value as previously determined. Using the bottom slider bar marked "var", set the variance to 1.22x10<sup>-4</sup>

STRmix Probabilistic Genotyping Software Operating Instructions					
Status: Published	Status: Published Document ID: 6482				
DATE EFFECTIVE	APPROVED BY	PAGE			
08/26/2021	Nuclear DNA Technical Leader	13 OF 40			

- 2.10.6.1.5 Using the drop down, change the contributor to number 2. Using the top slider bar marked "mean", set the mean to the value as previously determined. Using the bottom slider bar marked "var", set the variance to  $1.22 \times 10^{-4}$ .
- 2.10.6.1.6 Repeat the same process for contributor 3 (if needed).

\*\*\*Even though the variances are the same for each of the contributors, you must move the slider bar off the original value and back to it in order to ensure the value is set correctly.\*\*\*

- 2.10.6.1.7 Select "Done". This will return you to the Populations Settings window. Ensure the box marked "User informed Mx priors" is checked.
- 2.11 After selecting "Start", the "Calculation Progress" window will open showing the Burnin progress and Main MCMC Progress.

STRmix - Calculation Progress		×
Calculation Progress Calculation began at Fri Oct 28 11:46:16	EDT 2016	
MCMC 1: Locus 6 - 3 reasonable genotypes MCMC 1: Locus 7 - 1 reasonable genotypes MCMC 1: Locus 9 - 1 reasonable genotypes MCMC 1: Locus 9 - 1 reasonable genotypes MCMC 1: Locus 10 - 2 reasonable genotype MCMC 1: Locus 11 - 3 reasonable genotype MCMC 1: Locus 12 - 2 reasonable genotype MCMC 1: Locus 13 - 1 reasonable genotype MCMC 1: Locus 13 - 1 reasonable genotype MCMC 1: Locus 15 - 1 reasonable genotype MCMC 1: Locus 21 - 1 reasonable genotype MCMC 1: Locus 23 - 3 reasonable genotype MCMC 1: Locus 23 - 3 reasonable genotype MCMC 1: Locus 23 - 3 reasonable genotype MCMC 1: Locus 33 - 3 reasonable genotype MCMC 1: Locus 34 - 1 reasonable genotype MCMC 1: Locus 35 - 7 reasonable genot	3 3 3 3 3 3 3 3 3 3 3 3 3 3	4 III 4
Pre-Burnin Progress: All Loci complete (:	23 total)	
Burnin Progress:	Main MCMC Progress:	
STRmix V2.4.05 - User: kmckay	Cancel calculation View R	esults

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

FURENSIC E	SIULUGI PRUTUCULS FUR FURENSI	2 SIK ANALYSIS
STRmix Pr Status: Published	obabilistic Genotyping Software Opera	ting Instructions Document ID: 6482
DATE EFFECTIVE	APPROVED BY	PAGE
08/26/2021	Nuclear DNA Technical Leader	14 OF 40
STI	STRmix - Calculation Results  sults  sults  STRmix V2 4 05 - User - kmckay Analysis run: 2016/10/28 10 58.31 Case Number 57 Rmix Deconvolution Sample D: 1 Considering Evidence as originating from 2 individual(s)  PARAMETERS  Considering Evidence as originating from 2 individual(s)  Pre-Burnin time taken: 0.085 secs post Burnin time taken: 16.635 secs post Burnin time taken: 16.635 secs post Burnin time taken: 16.635 secs Soft Burni time taken: 16.635 secs Soft Burni time taken: 16.635 secs Soft Burni time taken: 16.635 secs Dost Burni time taken: 16.635 secs Soft Burni time taken: 16.635 secs Dost Burni time taken: 16.635 secs Dost Burni time taken: 16.635 secs Soft Burni time taken: 16.635 secs Dost Burni	

- 2.13 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
  - $\mathbf{\mathbf{x}}$

2.14

\*CAUTION: If you hit "Finish" a Run Report will NOT be generated, and the analysis will have to be re-done.



2.15 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.

STRmix Probabilistic Genotyping Software Operating Instructions				
Status: Published Document ID: 648				
DATE EFFECTIVE	APPROVED BY	PAGE		
08/26/2021	Nuclear DNA Technical Leader	15 OF 40		

- 2.16 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.
- 2.17 Navigate to your STRmix Run Folder within the "STRmix Fileshare" folder (\\csc\ocme\OCME\_STRmix\_Fileshare). COPY your run folder into the previously created FB sample folder within the "STRmix Data" folder. Once you have copied the folder, CONFIRM that all files for that run have transferred over correctly to the "STRmix Data" folder. After confirmation, the copy of the STRmix Run folder located in the "STRmix Fileshare" folder should be deleted.

# **3** Likelihood Ratio calculations with STRmix<sup>TM</sup>:

- 3.1 Note: 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.
- 3.2 **Launch the STRmix™ 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".



- 3.3 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.
- 3.4 The STRmix "Configure Analysis" window will open.

STRmix Probabilistic Genotyping Software Operating Instructions

Status: Published		Document ID: 6482
DATE EFFECTIVE	APPROVED BY	PAGE
08/26/2021	Nuclear DNA Technical Leader	16 OF 40

- 3.4.1 Rarely, a locus may be ignored at this step. For example, in the case of an unresolved peak or tri-allelic pattern that matches your reference sample, which was not recognized at the deconvolution stage:
  - 3.4.1.1 Click **Run Settings** at the bottom of the window.
  - 3.4.1.2 Under **Thresholds** settings click **Ignore Loci** button.
  - 3.4.1.3 Select the locus within the **Include** window and click the > button to move the locus to the **Exclude** window.
  - 3.4.1.4 Click save when all appropriate loci have been added to the **Exclude** window.
  - 3.4.1.5 Click save again in the **Run Settings** window.
- 3.5 Naming STRmix<sup>TM</sup> Likelihood Ratio runs
  - 3.5.1 The Case Number and Sample ID will auto-populate from the deconvolution settings file. **This should be updated to the appropriate naming convention for an LR run before proceeding.** The following naming convention should be used:
    - 3.5.1.1 Evidence File:

**Case Number** = YY-XXXXX (leave out "FB")

**Sample ID** = remainder of the evidence sample OCME ID\*\_scenario for LR **Case notes** = a comment should be added here if a locus is ignored, indicating the reason: e.g. "D2S441 was ignored due to an unresolved allelic peak"

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

3.5.1.2 Suspect File:

Case Number = SYY-XXXX (leave out "FB") Sample ID = OCME ID\* for evidence sample (leave out "FB")\_scenario for LR Case Notes = leave blank

STRmix Probabilistic Genotyping Software Operating Instructions					
Status: Published Document ID: 648					
DATE EFFECTIVE	APPROVED BY	PAGE			
08/26/2021	Nuclear DNA Technical Leader	17 OF 40			

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

<u>e.g.</u> 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

3.5.1.4 Note – 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
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	

- 3.6 Select "Confirm". Once "Confirm" is hit, a folder will be created in the STRmix results folder on the M 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.
- **3.7** 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".

FUREN	<b>SIU E</b>	DIULUGI I KUI	OCOLS FOR	FURI	STORE 2	I K ANAL I SIS
STRm Status: Published	ix Pr	obabilistic Gen	otyping Softw	vare (	Operatin	g Instructions Document ID: 6482
DATE EFFECTIV	Έ	AP	PROVED BY			PAGE
08/26/2021		Nuclear DN	IA Technical Le	ader		18 OF 40
	STRrip	mix - Add Profile Data 2: Add Evidence Profile Data Add Profile emove Profile teference Profile Data Add Profile teference Profile Data Add Profile teference Profile Data Add Profile teremove Profile teremove Profile teremove Profile teremove Profile	30-29M30_500pg_10-1_E0	Contrib Hp	a. csv	

A CV PROTOCOL C FOR FORENCIC CTR A NAL VOIC

- 3.8 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 step <u>3.7</u> for that comparison sample.
- 3.9 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.
- 3.10 Select "Confirm settings".
- 3.11 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.

FORENSI	C BIOLO	DGY PROT	OCOLS FO	R FORENSIC	STR ANALYSIS
STRmix Status: Published	Probabi	listic Gen	otyping So	ftware Operat	ing Instructions Document ID: 6482
DATE EFFECTIVE		AP	PROVED BY		PAGE
08/26/2021		Nuclear DN	IA Technical	Leader	19 OF 40
	1			ľ	
( a s	STRmix - Population	Settings		F	×
-Si	tep 3: Population Sett	inas			
			2.1.1		
		Add Population R	emove Population		
	Population	Proportion	FST	Allele Freq File	
	NIST_AfAm	0.25	0.03b(1.0,1.0)	NIST Fusion AfAm_Ame	
	NIST_Asian	0.25	0.03b(1.0,1.0)	NIST Fusion Asian_Ame	
	NIST_Cauc	0.25	0.03b(1.0,1.0)	NIST Fusion Cauc_Amen	
	NIST_Hisp	0.25	0.036(1.0,1.0)	NIST Fusion Hisp_Amen	
	ange	m 2 to 2 contributors butor # under Hp and Hd N! LR	Use informed Mx priors	#	
	ampling Variation — Calculate HPD HPD iterations: 1000	•	✓ Include MCMC unce Quantile: 99 ▼	ertainty Sides: 1 💌	
TIZ	Save as default Rmix V2.4.08 - User: 1	vasquez	Cancel Back	Start Start & Search	

- 3.12 Select "Start" to calculate the LR
- 3.13 The Calculation Progress screen will open, and the software will progress through burnin and Main MCMC Progress.
- 3.14 The STRmix calculations Results window will then open. Choose "Run Report"

3.15 **\*\*\*CAUTION: If you hit "Finish" a Run Report will NOT be generated, and the** analysis will have to be re-done.

- 3.16 The Advanced Report window will open. Select "Create Report".
- 3.17 The STRmix PDF report will open and will save in the relevant folder and then close the report.
- 3.18 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.

STRmix Probabilistic Genotyping Software Operating Instructions				
Status: Published Document ID: 6482				
DATE EFFECTIVE	APPROVED BY	PAGE		
08/26/2021	Nuclear DNA Technical Leader	20 OF 40		

3.19 Navigate to your STRmix Run Folder within the "STRmix Fileshare" folder (\\csc\ocme\OCME\_STRmix\_Fileshare). COPY your run folder into the previously created FB sample folder within the "STRmix Data" folder. Once you have copied the folder, **CONFIRM that all files for that run have transferred over correctly** to the "STRmix Data" folder. After confirmation, the copy of the STRmix Run folder located in the "STRmix Fileshare" folder should be deleted.

# 4 Deconvolution and Likelihood Ratio Calculations (Combined) in STRmix<sup>TM</sup>.

- 4.1 This option may be used under the following scenarios:
  - 4.1.1 Single source evidence sample that did not require a STRmix deconvolution for determination of a profile, needing an LR to a matching comparison sample
  - 4.1.2 Evidence mixture sample undergoing deconvolution and an LR check to determine if a reasonably expected reference sample can be used for further conditioning (ex. car owner on a swab from the steering wheel).
  - 4.1.3 To assess a probative comparison sample's presence within a mixture where that reference sample data is contained within the same evidence file (ex. victim's blood on suspect's clothing.)
- 4.2 **Launch the STRmix<sup>TM</sup> application.** Open the STRmix<sup>TM</sup> software by locating STRmix<sup>TM</sup> in the task bar or by double clicking on the STRmix<sup>TM</sup> icon on the desktop.
- 4.3 Select "Start Analysis" from the startup screen. This will open the "STRmix Configure Analysis" window.

STRmix Probabilistic Genotyping Software Operating Instructions         Date EFFECTIVE       APPROVED BY       PAGE         08/26/2021       Nuclear DNA Technical Leader       21 OF 40         STRmix Configure Analysis         Case Number         Sample ID       Case Number         Case Notes	FORENSIC I	IOLOGY FROTOCOLS FOR FOREISI	C SIN ANALISIS
DATE EFFECTIVE 08/26/2021       APPROVED BY Nuclear DNA Technical Leader       PAGE 21 OF 40         Image: STRmix - Configure Analysis       Image: STRmix - Configure Analysis       Image: STRmix - Configure Analysis         Image: STRmix - Configure Analysis       Image: STRmix - Configure Analysis       Image: STRmix - Configure Analysis         Image: STRmix - Configure Analysis       Image: STRmix - Configure Analysis       Image: STRmix - Configure Analysis         Image: Step 1: MCMC settings       Image: Step 1: MCMC settings       Image: Step 1: MCMC settings         Image: Number of contributors       Image: Step 1: MCMC settings       Image: Step 1: MCMC settings         Image: Number of contributors       Image: Step 1: MCMC settings       Image: Step 1: MCMC settings         Image: Number of contributors       Image: Step 1: MCMC settings       Image: Step 1: MCMC settings         Image: Number of contributors       Image: Step 1: MCMC settings       Image: Step 1: MCMC settings         Image: Number of contributors       Image: Step 1: MCMC settings       Image: Step 1: MCMC settings         Image: Step 1: MCMC accepts       Image: Step 1: MCMC settings       Image: Step 1: MCMC settings         Image: Step 1: MCMC accepts       Image: Step 1: MCMC settings       Image: Step 1: MCMC settings         Image: Step 1: MCMC settings       Image: Step 1: MCMC settings       Image: Step 1: MCMC settings         I	STRmix Pr	obabilistic Genotyping Software Opera	nting Instructions
08/26/2021       Nuclear DNA Technical Leader       21 OF 40         Image: STRmix - Configure Analysis       Image: STRmix: Case details       Image: STRmix: Case details         Sample ID       Image: Step 1: MCMC settings       Image: Step 1: MCMC settings         Number of contributors       Image: Step 1: MCMC settings       Image: Step 1: MCMC settings         Number of contributors       Image: Step 1: MCMC settings       Image: Step 1: MCMC settings         Image: Step 1: MCMC settings       Image: Step 1: MCMC settings       Image: Step 1: MCMC settings         Image: Step 1: MCMC settings       Image: Step 1: MCMC settings       Image: Step 1: MCMC settings         Image: Step 1: MCMC settings       Image: Step 1: MCMC settings       Image: Step 1: MCMC settings         Image: Step 1: MCMC settings       Image: Step 1: MCMC settings       Image: Step 1: MCMC settings         Image: Step 1: MCMC settings       Image: Step 1: MCMC settings       Image: Step 1: MCMC settings         Image: Step 1: MCMC settings       Image: Step 1: MCMC settings       Image: Step 1: MCMC settings         Image: Step 1: MCMC settings       Image: Step 1: MCMC settings       Image: Step 1: MCMC settings         Image: Step 1: MCMC settings       Image: Step 1: MCMC settings       Image: Step 1: MCMC settings         Image: Step 1: MCMC settings       Image: Step 1: MCMC settings       Image: Step 1: MCMC settings<	DATE EFFECTIVE	APPROVED BY	PAGE
STRmix - Configure Analysis     STRmix: Case details   Case Number   Sample ID   Case Notes     Step 1: MCMC settings   Number of contributors 1   DNA kit used   OCME_Fusion   # MCMC accepts   500000   # burnin accepts   100000     Run Settings   Cancel   Confirm	08/26/2021	Nuclear DNA Technical Leader	21 OF 40
STRmix - Configure Analysis     STRmix: Case details   Case Number   Sample ID   Case Notes     Step 1: MCMC settings   Number of contributors   I   DNA kit used   OCME_Fusion   # MCMC accepts   500000   # burnin accepts   100000     Run Settings   Cancel   Confirm			
STRmix: Case details   Case Number   Sample ID   Case Notes     Step 1: MCMC settings   Number of contributors   Number of contributors   DNA kit used   OCME_Fusion   # MCMC accepts   500000   # burnin accepts   100000     Run Settings   Cancel   Confirm	호 STRmix - Config	ure Analysis	×
Case Number Sample ID Case Notes Step 1: MCMC settings Number of contributors 1 DNA kit used OCME_Fusion • # MCMC accepts 50000 # burnin accepts 100000 Run Settings Cancel Confirm STRmix V2.4.05 - User: kmckay	_ STRmix: Case det	ils	
Sample ID Case Notes Step 1: MCMC settings Number of contributors 1 DNA kit used OCME_Fusion • # MCMC accepts 500000 # burnin accepts 100000 Run Settings Cancel Confirm STRmix V2.4.05 - User: kmckay	Case Number		
Case Notes	Sample ID		
Step 1: MCMC settings   Number of contributors   DNA kit used   OCME_Fusion   # MCMC accepts   500000   # burnin accepts   100000     Run Settings   Cancel   Confirm   STRmix V2.4.05 - User: kmckay	Case Notes		
Step 1: MCMC settings   Number of contributors   1   DNA kit used   OCME_Fusion   # MCMC accepts   500000   # burnin accepts   100000     Run Settings   Cancel   Confirm			
Step 1: MCMC settings   Number of contributors   DNA kit used   OCME_Fusion   # MCMC accepts   500000   # burnin accepts   100000     Run Settings   Cancel   Confirm   STRmix V2.4.05 - User: kmckay			
Step 1: MCMC settings         Number of contributors         DNA kit used         OCME_Fusion         # MCMC accepts         500000         # burnin accepts         100000         Run Settings         Cancel         Confirm         STRmix V2.4.05 - User: kmckay			
Number of contributors       1         DNA kit used       OCME_Fusion         # MCMC accepts       500000         # burnin accepts       100000         Run Settings         Cancel       Confirm         STRmix V2.4.05 - User: kmckay	Step 1: MCMC set	ings	
DNA kit used OCME_Fusion  # MCMC accepts 500000 # burnin accepts 100000 Run Settings Cancel Confirm STRmix V2.4.05 - User: kmckay	Number of contr	putors 1	
# MCMC accepts       500000         # burnin accepts       100000         Run Settings       Cancel         Confirm         STRmix V2.4.05 - User: kmckay	DNA kit used	OCME_Fusion	
# burnin accepts 100000 Run Settings Cancel Confirm STRmix V2.4.05 - User: kmckay	# MCMC accepts	500000	
Run Settings Cancel Confirm STRmix V2.4.05 - User: kmckay	# burnin accepts	100000	
Run Settings Cancel Confirm			
STRmix V2.4.05 - User: kmckay		Run Settings Cancel	Confirm
	STRmix V2.4.05 - U	er: kmckay	

EADENSIC DIALACY DUATACALS EAD EADENSIC STD ANALYSIS

- 4.4 Naming STRmix<sup>TM</sup> runs
  - 4.4.1 STRmix<sup>™</sup> 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.
  - 4.4.2 The following naming convention should be used for deconvolution and LR combined runs:
    - 4.4.2.1 Evidence Files

**Case Number** = YY-XXXXX (leave out "FB") **Sample ID** = remainder of the evidence sample OCME ID\* #NOC scenario for LR **Case notes** = a comment should be added here if a locus is ignored, indicating the reason: e.g. "D2S441 was ignored due to an unresolved allelic peak"

e.g. decon and 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 2p JD1Uv2U

STRmix I	Probabilistic	Genotyping	Software (	Operating	Instructions
	1000001110010		Solution	per anno	

Status: Published		Document ID: 6482
DATE EFFECTIVE	APPROVED BY	PAGE
08/26/2021	Nuclear DNA Technical Leader	22 OF 40

#### 4.4.2.2 Suspect Files

**Case Number** = SYY-XXXXX (leave out "FB")

**Sample ID** = OCME ID\* for evidence sample (leave out "FB") #NOC scenario for LR

**Case Notes** = a comment should be added here if a locus is ignored, indicating the reason: e.g. "D2S441 was ignored due to an unresolved allelic peak"

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

<u>e.g.</u> 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 3p TS2Uv3U

4.4.2.4 Note – 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	
Single source, elimAB LR	16-01234-567-1-1-1-shirt-BL-1p-ABv1U
2-person mixture, elimAB LR	16-01234-567-1-1-1-shirt-BL-2p-AB1Uv2U
3-person mixture, conditioned CD, elim AB LR	16-01234-567-1-1-1-shirt-BL-3p-ABCD1UvCD2U
Suspect File	
Single source, suspTS LR	S16-05678-16-01234-567-2-1-1-slide-GS-1p-TSv1U
2-person mixture, suspTS LR	S16-05678-16-01234-567-2-1-1-slide-GS-2p-TS1Uv2U
2-person mixture, conditioned CD, susp TS LR	S16-05678-16-01234-567-2-1-1-slide-GS-2p-TSCDvCD1U
3-person mixture, conditioned AB, suspTS LR	S16-05678-16-01234-567-2-1-1-slide-GS-3p-
	TSAB1UvAB2U

- 4.5 Set the number of contributors.
- 4.6 Ensure that the following "Step 1: MCMC settings" are in place:

DNA kit used: OCME\_Fusion # MCMC accepts: 500000 # burnin accepts: 100000

4.7 NOTE: the # MCMC accepts and # burnin accepts must not be modified without documented approval from the technical leader (or his/her designee).

STRmix Probabilistic Genotyping Software Operating Instructions

Status: Published		Document ID: 6482
DATE EFFECTIVE	APPROVED BY	PAGE
08/26/2021	Nuclear DNA Technical Leader	23 OF 40

- 4.8 Select "run settings" to confirm run settings. The settings should be as follows for every STRmix<sup>TM</sup> analysis. Any changes that are made will appear in bold on the run report. Press "Cancel" when done.
  - 4.8.1 If a locus needs to be ignored for the deconvolution:
    - 4.8.1.1 Under **Thresholds** settings click **Ignore Loci** button.
    - 4.8.1.2 Select the locus within the **Include** window and click the > button to move the locus to the **Exclude** window.
    - 4.8.1.3 Click save when all appropriate loci have been added to the **Exclude** window.
    - 4.8.1.4 Click save again in the **Run Settings** window.

MM	STRmix - Run Settings			X
	Run Settings			
	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 Degradation r	nax	мсмс	
	-1.0 Degradation s	starts at (bp)	8 Number of chains	
	Drop-in		Low Memory Mode	
	100.0 Drop-in cap		Extended Output	
	0.0024 Drop-in frequ	ency	9.0 Post burn-in shortlist	
	0.0,0.0 Drop-in gamm	na parameters	0.005 Random Walk SD	
			Seed	
			Random 948367	
H			Cancel	ave
	STRmix V2.4.05 - User: kmc	kay		

4.9 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 the M drive. If incorrect nomenclature was used and you

STRmix Probabilistic Genotyping Software Operating Instructions				
Status: Published Document ID: 6482				
DATE EFFECTIVE	APPROVED BY	PAGE		
08/26/2021	Nuclear DNA Technical Leader	24 OF 40		

return to this screen to make changes, the empty folder on your STRmix results drive should be deleted.

- 4.10 Add your Evidence Profile Data.
  - **4.10.1** In the "STRmix Add Profile Data" window, select "Add Profile". This will bring you to the "Adding Evidence Profile Data" window (see step 4.10.2 below) where you can select either a text file or STRmix file from which to run a STRmix 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 4.10.4.

∑ STRmix - Add Profile Data	×
Step 2: Add Evidence Profile Data	
Add Profile	
Remove Profile	
- Add Reference Profile Data	
	Contributor to:
Add Profile	
Remove Profile	
Change Hd	
Cancel Back	Confirm settings
STRmix V2.4.05 - User: Kmckay	

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

FU	KEN2IC R	IOLOGY PROTOCOLS I	FOR FORENSI	<u>C SIR AP</u>	ALY 515
Status: Published	TRmix Pr	obabilistic Genotyping S	Software Opera	ating Inst	ructions
DATE EFFE	ECTIVE	APPROVED E	3Y	I	PAGE
08/26/20	021	Nuclear DNA Technic	cal Leader	25	OF 40
MA	Adding Eviden Step 2: Add Evid Profiling Kit ii Import from Chosen File: Chosen sample: Export filename Choose previ Chosen File:	ce Profile Data ence Profile Data ence Profile Data use: OCME_Fusion plate text file: (s): Chosen sample(s) from file (s): Name(s) to save sample(s) as ous STRmix Evidence file: Jser: kmckay	Alleles per loc Edit Select STR Cancel Add I	ext File cus: 15 Rmix File Profile Data	

PROTOCOL C FOR FORENCIC CTR ANALYCIC

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

M	Choose Profile Sampl
Ir	Choose Profile(s)
	01-Allelic_Ladder_1_A01_001.f
	05-M2_C2_500_2-1_15M_5F_E 06-M2_C2_500_1-1_15M_5E_E
	< Ⅲ ►
	Cancel Add

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

FORENSIC B	<b>BIOLOGY PROTOCOLS FOR FORENSI</b>	C STR ANALYSIS
STRmix Pr Status: Published	obabilistic Genotyping Software Opera	ting Instructions
DATE EFFECTIVE	APPROVED BY	PAGE
08/26/2021	Nuclear DNA Technical Leader	26 OF 40
<ul> <li>➢ Adding</li> <li>Step 2: A</li> <li>Profiling</li> <li>Import</li> <li>Chosen</li> <li>Export fi</li> <li>Choosee</li> <li>Chosen</li> </ul>	Evidence Profile Data dd Evidence Profile Data g Kit in use: OCME_Fusion from plate text file: File: Newton041916 11 Experiment 8.txt sample(s): 06-M2_C2_500_1-1_15M_5F_F01_011.fsa. Alleles per locu lename(s): 06-M2_C2_500_1-1_15M_5F_F01_011.fsa. Edit previous STRmix Evidence file: File: Cancel Add Profile 4.05 - User: kmckay	

**4.10.6** Now select "Add Profile Data" and your text file name will appear in the "Add profile Data" screen as seen below. Repeat steps <u>4.10.1 - 4.10.6</u> to add any replicates of the sample which were amplified.

FURENSIC E	DIOLOGY FROTOCOLS FOR F	UKENSI	C SIN ANALISIS
STRmix Pr	obabilistic Genotyping Softwa	re Opera	ating Instructions
DATE EFFECTIVE	APPROVED BY		PAGE
08/26/2021	Nuclear DNA Technical Load	dor	27 OF 40
08/20/2021	Nuclear DNA Technicar Lea	uei	27 01 40
▼    STRmix      Step 2: Add      Add      Remo	- Add Profile Data dd Evidence Profile Data I Profile 06-M2_C2_500_1-1_15M_5F_F01_03 ve Profile 0ata ence Profile Data I Profile ve Profile 0ata Inge Hd Cancel Back 4.05 - User: kmckay	Contributor Hp Ho	to: d ettings

EADENSIC DIALACY DUATACALS EAD EADENSIC STD ANALYSIS

- 4.11 **Deconvolutions without a conditioned contributor**. Proceed to Step 4.13.
- 4.12 **Deconvolutions with a conditioned contributor**: 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.
  - 4.12.1 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 <u>4.12.3</u>.
  - 4.12.2 Click on "Select Text F..." to navigate to the case conditioned reference text file.
  - 4.12.3 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.
  - 4.12.4 The conditioned sample will now appear in the "Add Reference Profile Data" section of the screen below.

FURENSIC	BIOLOGY PROTOCOLS FOR FORENSIG	SIK ANALYSIS
STRMIX P	robabilistic Genotyping Software Opera	ting Instructions
DATE FEFECTIVE	APPROVED BY	PAGE
08/26/2021	Nuclear DNA Technical Londor	28 OF 40
08/20/2021	Nuclear DNA Technical Leader	28 01 40
	STRmix - Add Profile Data          Step 2: Add Evidence Profile Data         Add Profile       07-M1_C1_750_1-2_6M_22F_G01_013.fsa.csv         Remove Profile       07-M1_C1_750_1-2_6M_22F_G01_013.fsa.csv         Add Reference Profile Data       Contributor to:         Add Profile       20-22F_D03_007.fsa.csv         Kemove Profile       X         Change Hd       Confirm settings	
	STRmix V2.4.05 - User: kmckay	

A CV PROTOCOL C FOR FORENCIC CTR A NAL VOIC

4.12.5 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.

STRmix - Add Profile	e Data		
Step 2: Add Evidence Pr	rofile Data		וו
Add Profile	07-M1_C1_750_1-2_6M_22F_G0	01_013.fsa.csv	
Remove Profile			
Add Reference Profile D	ata	Contributor to:	
		Нр Hd	1
Add Profile	20-22F_D03_007.fsa.csv	x x	
Remove Profile			
Change Hd			
	Coursel Day		
CTD min V2 4 05 - Upper l	Cancel Bac	Confirm settings	
STRMIX V2.4.05 - User: k	ктскау		

4.13 Add appropriate reference samples that need an LR calculated. Each sample that is added will be within the numerator of the LR (ie. assigned to H<sub>p</sub>).

STRmix Probabilistic Genotyping Software Operating Instructions			
Status: Published Document ID: 6482			
DATE EFFECTIVE	APPROVED BY	PAGE	
08/26/2021	Nuclear DNA Technical Leader	29 OF 40	

4.14 Select "Confirm Settings". This will open up the Populations Settings window. Ensure that the following four populations are listed: NIST Caucasian, NIST Asian, NIST AfAm, and NIST Hisp and that the settings appear as below.

NIST_AfAm 👻	Add Population R	emove Population	
Population	Proportion	FST	Allele Freq File
NIST_AfAm	0.25	0.03b(1.0,1.0)	NIST Fusion AfAm_Ame
NIST_Asian	0.25	0.03b(1.0,1.0)	NIST Fusion Asian_Ame
NIST_Cauc	0.25	0.03b(1.0,1.0)	NIST Fusion Cauc_Amen
NIST_Hisp	0.25	0.03b(1.0,1.0)	NIST Fusion Hisp_Amen
ange	om 2 to 2 contributors tributor # under Hp and Hd	⊡ Stratify contributor	#
ange	om 2 to 2 contributors tributor # under Hp and Hd	Stratify contributor	#
ange	om 2 to 2 contributors tributor # under Hp and Hd FNI LR	Use informed Mx priors -	#
ange	om 2 to 2 contributors tributor # under Hp and Hd FN! LR	User informed Mx priors	# riors
ange Profiles originates fr Use MLE for cont actor N! Display Factor of ampling Variation Q Calculate HPD	om 2 to 2 contributors tributor # under Hp and Hd FNI LR	Use informed Mx priors -	# riors

- 4.15 After selecting "Start", the "Calculation Progress" window will open showing the Burnin progress and Main MCMC Progress.
- 4.16 When the analysis is complete, the raw data report will open as follows:

FORENSIC I	SIOLOGY PROTOCOLS FOR FORE	NSIC STR ANALYSIS
STRmix Published	obabilistic Genotyping Software Op	perating Instructions
DATE EFFECTIVE	APPROVED BY	PAGE
08/26/2021	Nuclear DNA Technical Leader	30 OF 40
00/20/2021	Tuclear DTVT Teeninear Deader	
Results- Results- TTRM Analy Seed PARA Consi Pre-B Burnin post E Total 1121/ Effect 15767 AST Burnin Post B Samp PARA Consi Pre-B Burnin Post B Samp PARA Consi Pre-B Burnin Post B Samp Para Burnin Post B Samp Burnin Post B Samp Burnin Post B Samp Burnin Post B Samp Burnin Post B Samp Burnin Post B Samp Burnin Burnin Post B Samp Burnin Burnin Burnin Burnin Post B Samp Burnin B	x - Calculation Results	

TOCOLO

NOTO OTD ANAL TROTO

- 4.17 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
- 4.18 \* CAUTION: If you hit "Finish" a Run Report will NOT be generated, and the analysis will have to be re-done.



4.19 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.

STRmix Probabilistic Genotyping Software Operating Instructions				
Status: Published Document ID: 6482				
DATE EFFECTIVE	APPROVED BY	PAGE		
08/26/2021	Nuclear DNA Technical Leader	31 OF 40		

- 4.20 Close out of the Advanced Report window and then select "Finish" on the "STRmix<sup>TM</sup> Calculation Results" window. This will return you to the STRmix<sup>TM</sup> start up screen.
- 4.21 Navigate to your STRmix Run Folder within the "STRmix Fileshare" folder (\\csc\ocme\OCME\_STRmix\_Fileshare). COPY your run folder into the previously created FB sample folder within the "STRmix Data" folder. Once you have copied the folder, CONFIRM that all files for that run have transferred over correctly to the "STRmix Data" folder. After confirmation, the copy of the STRmix Run folder located in the "STRmix Fileshare" folder should be deleted.

# 5 How to Run STRmix<sup>TM</sup> using Batch Mode

- 5.1 A number of STRmix<sup>™</sup> deconvolutions can be set up and queued to run sequentially. Note, if STRmix<sup>™</sup> cannot model the data by the chosen NOC, the batch mode will stop and <u>no data will be saved</u>.
- **5.2** Before setting up a batch, navigate to the "STRmix Fileshare" folder (<u>\\csc\ocme\OCME\_STRmix\_Fileshare</u>). Create a new folder within the "STRmix Fileshare" folder with the name of "Batch Mode [date] [your initials]."
- **5.3** To set up a queued analysis for multiple samples, select Batch Mode from the STRmix<sup>TM</sup> main window.

STRMIX. RESOLVE MORE DNA MIXTURES.		
Start Analysis	Settings	
LR from Previous Analysis	Model Maker	
Search Database	Batch Mode	1
	Exit	
STRmix V2.4.05 - User: kmckay	About	

5.4 Select "Change Batch Directory" and navigate to and select the created folder inside the "STRmix Fileshare." This new folder will now appear at the bottom left of the sample summary window as the directory.

#### FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS STRmix Probabilistic Genotyping Software Operating Instructions Status: Published Document ID: 6482 DATE EFFECTIVE APPROVED BY PAGE 32 OF 40 08/26/2021 Nuclear DNA Technical Leader 🖉 STRmix - Batch Mode Progress × Batch Mode Samples in Batch Add to Batch Plugin Configuration Delete analysis Start Batch Stop Batch Change batch directory Exit Batch Mode . \\csc\ocme\OCME\_STRmix\_Fileshare\Batch Mode [date] [initials] STRmix V2.4.08 - User: coconnor

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

	🖉 STRmix - Batch Mode Progress			×
$\frown$	Batch Mode			
	Add to Batch Plugin Configuration Delete analysis Start Batch Stop Batch Exit Batch Mode (rsoc/ocme/OCME_STRmix_Fileshare	th directory		
	STRmix V2.4.08 - User: coconnor			

- 5.6 Complete the analysis set up for the first sample following Section <u>2 Deconvolutions in</u> <u>STRmixTM.</u>
- 5.7 In the Population Settings window, select "Start" to return to the Batch Mode window.
- 5.8 In the Batch Mode Window, select "Add to Batch" to enter the next sample. Repeat steps 5.4 5.7 to add additional samples.
  - 5.8.1 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".
- 5.9 Select "Start Batch" to start the batch run.

STRmix Probabilistic Genotyping Software Operating Instructions

Status: Published		Document ID: 6482
DATE EFFECTIVE	APPROVED BY	PAGE
08/26/2021	Nuclear DNA Technical Leader	33 OF 40

- 5.10 After completion of analyses, select "Exit Batch Mode" to return to the STRmix<sup>TM</sup> main window.
- **5.11** Results folders from Batch Mode will be saved in the created folder inside of the "STRmix Fileshare" folder (<u>\\csc\ocme\OCME\_STRmix\_Fileshare</u>). Move the results folders from the batch mode folder to the appropriate FB folders within the STRmix Data drive. Once confirming that all files for the batch mode runs have been transferred over correctly, DELETE the created batch mode folder from the fileshare.

# **6** Evaluation of the STRmix<sup>TM</sup> Analysis

- **6.1** Verify the evidence and reference input sections of the STRmix<sup>TM</sup> printout against the associated electropherograms. Ensure that:
  - 6.1.1 All appropriate edits were made, no artifact peaks were left labeled
  - 6.1.2 No stutter (for evidence samples) or allelic peaks have been removed
  - 6.1.3 Correct input file(s) have been selected
  - 6.1.4 All suitable replicates have been utilized
    - 6.1.4.1 Check the "Inter replicate efficiency" that is generated by STRmix<sup>TM</sup>. If there are drastic inconsistencies between the two efficiencies that are consistent with the amount of data present in the replicates, the STRmix analysis may be marked as 'not reported' and a new analysis may be performed with the amplification with the most information. A third amplification may be warranted.
  - 6.1.5 The correct settings file was imported into an LR from previous analysis, if applicable
- 6.2 The number of contributors that best describes the data has been chosen
- 6.3 The correct assumptions (conditioning) have been made, if applicable
- 6.4 The appropriate proposition has been selected (LR calculation), if applicable
- 6.5 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).

# STRmix Probabilistic Genotyping Software Operating Instructions

Status: Published		Document ID: 6482
DATE EFFECTIVE	APPROVED BY	PAGE
08/26/2021	Nuclear DNA Technical Leader	34 OF 40

#### 6.6 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
Stutter					
Forward Stutter	0.1	Maximum	0.01	Burn-in Accepts	100000
Max		Degradation			
Excluded Loci	DYS391	Saturation	8000	Chains	8

STRmix Probabilistic Genotyping Softwa	re Operating Instructions
--	---------------------------

Status: Published		Document ID: 6482
DATE EFFECTIVE	APPROVED BY	PAGE
08/26/2021	Nuclear DNA Technical Leader	35 OF 40

#### 6.7 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

- 6.8 The seed value is the starting number used within the random number generator. For deconvolutions and combined deconvolution/likelihood ratio calculations, the seed value is listed in the Run Information table on the first page of the report PDF. For likelihood ratio calculations, the seed value is listed in the Results text file within the run folder.
- 6.9 Verify that the following (**primary**) diagnostics conform to your qualitative expectations when compared to the electropherogram(s):
  - 6.9.1 The **mixture proportions** and **template amounts** assigned to the contributor(s)
  - 6.9.2 The **weights** assigned to the genotypes for each contributor listed in the Genotype Probability Distribution
  - 6.9.3 The degradation values and Locus Efficiencies (LSAE)
- 6.10 Evaluate the following (secondary) diagnostics for the run information.
  - 6.10.1 **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 accepts. If the total iterations approaches or exceeds 2.15 billion (2.15 x 10<sup>9</sup>), this may lead to incorrect genotype weightings being assigned.

STRmix Probabilistic Genotyping Software Operating Instructions

Status: Published		Document ID: 6482
DATE EFFECTIVE	APPROVED BY	PAGE
08/26/2021	Nuclear DNA Technical Leader	36 OF 40

- 6.10.2 **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 ESS value (tens or hundreds) means that there is potential for a large difference in weights if the analysis is run again.
- 6.10.3 **Average (log) likelihood.** The larger this value, the better STRmix<sup>TM</sup> has been able to describe the observed data. A negative value suggests that STRmix<sup>TM</sup> 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 log<sub>10</sub> (likelihood) may indicate to users that the analysis requires additional scrutiny.
- 6.10.4 **Gelman-Rubin diagnostic.** If this value is above 1.2 then it is possible that the analysis has not converged.
- 6.10.5 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.
- 6.11 For LR comparisons: The overall **category of support** (inclusion, uninformative, exclusion) should conform to your qualitative expectations in comparison to the data. Evaluate the **Per** Locus Likelihood Ratio table per locus and per sample, as well as the range of LR's between population subgroups; pay special attention to outliers and/or zero values.
- **6.12** For LR comparisons resulting in support for an inclusion: check to ensure that the comparison sample falls in the appropriate "Contributor Order giving highest LR". If multiple comparison samples are positively associated with the same sample, results should be evaluated to ensure that they do not align with the same contributor.

# 7 Troubleshooting Guide

The presence of a single sub-optimal diagnostic is not always an indication that rework is required. In some instances a sub-optimal diagnostic(s) may be due to the nature of the sample (ex. low amounts of input DNA and/or stochastic effects), and not due to an issue with the STRmix<sup>TM</sup> run. Refer to the Troubleshooting Guide below for further steps that may be taken to improve a sub-optimal diagnostic result.

The purpose of this guide is to address commonly seen scenarios which arise in casework. These guidelines are based on validation studies, literature references, and casework experience. However, not every situation can be covered by the Troubleshooting Guide. If a diagnostic issue arises that is not covered here, please discuss the issue with your supervisor, manager or the Technical Lead Team as needed.

STRmix Probabilistic Genotyping Software Operating Instructions

Status: Published		Document ID: 6482
DATE EFFECTIVE	APPROVED BY	PAGE
08/26/2021	Nuclear DNA Technical Leader	37 OF 40

Observation	Action
The mixture proportions or template amount do not reflect what is observed AND/OR the degradation does not reflect what is observed AND/OR the interpreted contributor genotypes do not conform to your qualitative expectations AND/OR the category of support for an LR comparison does not conform to your qualitative expectations.	Re-evaluate the number of contributors; consider another STRmix <sup>TM</sup> analysis with one higher or one lower number of contributors
	Consider amplifying a replicate if one has not already
	been done, with increased input amount when
	available and appropriate
	Inhibition has occurred—microcon to clean and reamplify sample
	Consider another STRmix <sup>TM</sup> analysis at greater
	number of accepts (typically, 1,000,000 burn-in
	accepts and 5,000,000 total accepts). Note: this
	requires approval by the Technical Leader (or his/her designee)
For a two or three person mixture with an	Consider another STRmix analysis utilizing informed
extreme ratio: ex. 98:2 or 99:1; the mixture	(Mx) priors. This requires approval by the Technical
proportions and genotype weights do not	Leader (or his/her designee).
conform to your qualitative expectations based	
on the electropherograms, and other trouble-	
shooting options (including additional	
iterations) have been exhausted.	
A low or negative average (log) likelihood	Reevaluate number of contributors; consider another
	STRmix <sup>IM</sup> 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
Column Dubin reduction and the state	removed
Geiman-Kubin value is greater than 1.2	Consider another STRMIX <sup>194</sup> analysis at greater
	number of accepts (typically, 1,000,000 burn-in
	requires approval by the Technical Leader (or his/her
	designee)
	ucorgroup

STRmix Probabilistic Genotyping Software Operating Instructions

Status: Published		Document ID: 6482
DATE EFFECTIVE	APPROVED BY	PAGE
08/26/2021	Nuclear DNA Technical Leader	38 OF 40

Stutter and/or allele variance significantly	Check to make sure no data has been omitted
with low average (log) likelihood)	
	Reevaluate number of contributors; consider another STRmix <sup>™</sup> analysis with one higher or one lower number of contributors
	Consider amplifying a replicate if one has not already been done, with increased input amount when available and appropriate
Large LR's (>1) obtained for each locus, except one where the LR = 0 and the POI reference is consistent with the evidentiary profile	Data entry problem—check input files
	Allele call not fully resolved at a given locus – ignore locus and perform analysis again. <b>Note:</b> 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, with increased input amount when available and appropriate
	Reevaluate number of contributors; consider another STRmix <sup>TM</sup> analysis with one higher or one lower number of contributors
	Consider another STRmix <sup>™</sup> analysis at greater number of accepts (typically, 1,000,000 burn-in accepts and 5,000,000 total accepts). <b>Note:</b> this requires approval by the Technical Leader (or his/her designee)
An error occurred  An error occurred while executing the analysis - Calculation failed:  Pre-Burnin Determine Genotypes failed: Locus 8 in the evidence cannot be explained given the parameters you have chosen	The number of contributors selected was too few and must be increased
ОК	

STRmix Probabilistic Genotyping Software Operating Instructions		
Status: Published		Document ID: 6482
DATE EFFECTIVE	APPROVED BY	PAGE
08/26/2021	Nuclear DNA Technical Leader	39 OF 40

Г

	Consider amplifying a replicate if one has not already
	been done, with increased input amount when
	available and appropriate
The STRmix <sup>™</sup> run does not progress at the	Check "alleles per locus" setting is set to 15.
MCMC burn in stage and the chains do not	
move. Error message also received that	
Degradation started at "0".	
An error occurred	"Start & Search" was clicked instead of "Start".
An error occurred while executing the analysis - empty String	Analysis must set up and performed again.
ОК	
The STR mix <sup>TM</sup> run does not progress past the	The number of contributors selected was too few and
MCMC hurn in stage and the log likelihood are	must be increased
listed at Infinity and/or NaN	must be moreused.
	One of the results files (GenotynePDF) from your
Calculation Progress	deconvolution is corrupted or missing and you are
Calculation began at Wed Jan 18 15:53:27 EST 2017	trying to run an LR from previous analysis Check
Copying file M/FBIOLOGY_MAIN/TRAINING/Analyst Folders/GeneMarker-STRmix Fc + Copying file C/ProgramData/STRmix/alleleFreqVNIST Fusion AfAm.csv to C/Program	your STRmix run folder for the associated
Copying file C:\ProgramData\STRmix\alleleFreq\VIST Fusion Asian.csv to C:\Program Copying file C:\ProgramData\STRmix\alleleFreq\VIST1036_strmix_Caucasian_FUS	deconvolution to check that all files are present
Number Of Loci = Message	Additionally check the input text files for your
3 to 3 How many knowr	samples ensure all values are present and aligned
2 What is the name 09-12F_A02_002	appropriately with their respective columns and are
What is the name 18-13M_B03_003.fsa.csv	match the inputs on the STRmix report. If the error
How many known contributors in Hp? 3 What is the name of the file that holds the reference of guestioned contributor 1?	persists the deconvolution may need to be re-run
09-12F_A02_002 fsa.csv What is the name of the file that holds the reference of questioned contributor 2 ?	
18-13M_B03_003.tsa.csv What is the name of the file that holds the reference of questioned contributor 3 ? 10-6M_B02_004 fsa.csv	
1 What is the name of the file that holds the genotype pdf?	
· · · · · · · · · · · · · · · · · · ·	
Multiple elimination and/or comparison samples	Consult a supervisor and the Tech Lead Team to go
that are positively associated with an evidence	over case specifics. Additional deconvolution and/or
sample are aligning with the same contributor	LR scenarios may need to be considered.
and/or there is an indication of relatedness.	
The 99% 1-sided HPD and Unified LR result(s)	Consult your supervisor or manager and the
for one or more population subgroup(s) drops	Technical Lead Team. A stratified likelihood ratio
several orders of magnitude lower than the other	using census data may be calculated and reported -
population subgroups in comparison to the point	this must be approved by the Technical Leader (or
estimate LR, and the lowest LR of the four	his/her designee).

STRmix Probabilistic Genotyping Software Operating Instructions

Status: Published		Document ID: 6482
DATE EFFECTIVE	APPROVED BY	PAGE
08/26/2021	Nuclear DNA Technical Leader	40 OF 40

population subgroups does not conform to your	
qualitative expectations of the comparison.	
The total iterations is approaching or exceeds	This could indicate that genotype weightings have
2.15 billion (2.15 x 10 <sup>9</sup> ).	been incorrectly assigned. Please contact the
	Technical Lead Team. An additional STRmix <sup>™</sup>
	analysis may be required.

# 8 **References:**

- 8.1 STRmix<sup>TM</sup> v.2.4 Operation Manual
- 8.2 STRmix<sup>TM</sup> v. 2.4 Users Manual