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**Operating instructions for MixtureCalc v1.2
(Freeware Version)**

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1   Objective

To provide instructions for importing data into and for performing mixture calculations using MixtureCalc-v1.2 (Freeware Version).

MixtureCalc-v1.2 (Freeware Version) is validated for SPSA casework only and no warranty is provided for non-SPSA use.

2   References

2.1   GeneMapper ID Software Version 3.1 User Guide (Controlled Document BIO-053).

3   Equipment

Computer with at Microsoft Excel (2000 or later) and Applied BioSystems GeneMapper ID 3.1.

4   Procedure

4.1   General

4.1.1 MixtureCalc –v1.2 allows the user to:

- Datagrab data from an exported GeneMapper file

- Allow raw or inputted data to be stutter adjusted or peak adjusted

- Calculation of mixture options

- Data sheets can be printed and calculations sheets saved

- Production of calculation sheets for LR calculations for Contributor1 and Contributor2

- LR Calculation

- Match Probability and Conditional Match Probability Calculations

5.1 Use of MixtureCalc-v1.2 Program

5.1.1 Open MixtureCalc and select to peak adjust or datagrab.

5.1.2 Press **NO** to go straight to mixture deconvolution sheet ("**FINAL**") (see **5.4**).

5.1.3 Press **YES** to datagrab (see **5.2**) or peakadjust (see **5.3**).

5.1.4 Select Manual input or datagrab.

5.2 Datagrab

5.2.1 Datagrab function will allow mixture data to be retrieved from exported GeneMapper data (see Appendix 1 for GeneMapper Settings).


5.2.2 Find and open exported GeneMapper data file.

5.2.3 Select and copy mixture data by highlighting the eleven rows (based on PCR number).

0	110684	7146A+B.(	D3S1358	15	16	17				722	371	295
1	110684	7146A+B.(	vWA	14	16					160	630	
2	110684	7146A+B.(	D16S539	10	11	12				180	295	192
3	110684	7146A+B.(	D2S1338	17						606		
4	110684	7146A+B.(	AMEL	X	Y					884	950	
5	110684	7146A+B.(	D8S1179	10	13	14				181	120	503
6	110684	7146A+B.(	D21S11	28	31.2	33.2				149	100	410
7	110684	7146A+B.(	D18S51	10	15	17				224	223	319
8	110684	7146A+B.(	D19S433	12	14	16				314	545	195
9	110684	7146A+B.(	TH01	6	7	8				26	516	557
0	110684	7146A+B.(	FGA	22	23	24				35	325	354

5.2.4   In MixtureCalc-v1.2 paste in Cell A9 in "DATAGRAB" table using Paste Special (values).
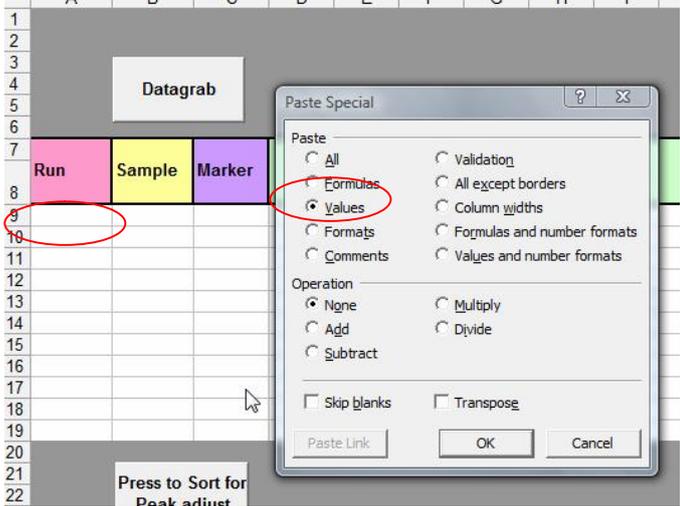
5.2.5   If data is to be either peak adjusted or stutter adjusted press [Press to Sort for Peak adjust] to transfer data to PeakAdjustment Sheet or press [Press to sort and tranfer to Mixture Calc] to transfer data to mixture sheet ("FINAL").

5.3   Peak Adjustment

5.3.1   Data can be entered manually into fields in bright yellow.

5.3.2   Check stutter values are set for current values, adjust if appropriate

5.3.3   Press Stutter Adjust button to highlight any stutter peaks, stutter peaks will not be transferred to mixture sheet.

LOCUS	Allele	Peak Height	Stutter Adjusted Peak Height	Stutter
D19	13	45	0	Stutter
	14	545	545	
	16	195	195	
	17	314	314	

5.3.4 Press Peak Adjust button to adjust for -8bp, -4bp and +4bp stutter in all peaks, stutter peaks will not be transferred to mixture sheet.

LOCUS	Allele	Peak Height	Adjusted Peak Height	Stutter
D19	12	26	-51	Stutter
	13	516	432	
	14	557	547	

5.3.5 Press Print button to get copy of adjusted data.

5.3.6 Press Transfer to MixCalc button to transfer adjusted data to mixture sheet ("FINAL").

5.4 Mixture Sheet ("FINAL")
5.4.1 If you select **NO** for peak adjust (see 5.1.2) you will be prompted to retrieve a saved sheet. Just press **NO** if you haven't saved.
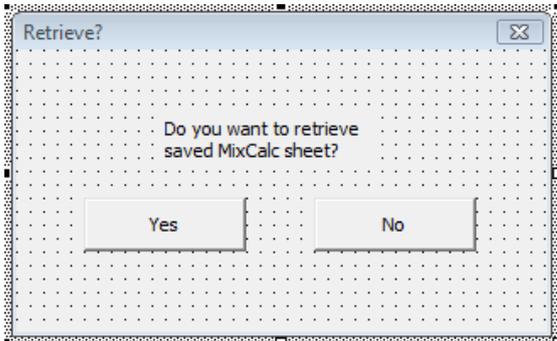

5.4.2 Press [Press to save sheet for later] to save mixture sheet by at anytime and save file in appropriate drive/folder.

5.4.3 Retrieve saved sheet by opening MixtureCalc program and pressing [Retrieve saved sheet]

5.4.4 Mixture sheet will display possibility of dropout depending on the number of peaks falling below heterozygous threshold.

Possibility of Dropout
YES
Estimated Profile Status
RED
RO Entered Status
RED

5.4.5   Select status profile by typing RED, AMBER or GREEN in box. Mixture sheet will assess profile status by calculating average peak height and selecting category.

Status	Ave Peak Height	Estimated Reciprocal Swing	Mx Tolerances
Green	>600	0.45-0.55	+/- 0.20
Amber	400-600	0.4-0.6	+/- 0.25
Red	<400	0.35-0.65	+/- 0.35

5.4.6   Select level of reciprocal swing. If not selected will default to level set by profile status estimate. The ↔ in the reciprocal column highlights reciprocal options. Delete this reciprocal if conditioning.

5.4.7   Check Heterozygous balance and Homozygous threshold

5.4.8   Mixture proportion (Mx) and Mixture Ratio (MR) are calculated and tolerance levels displayed depending on status profile.

5.4.9   Press calculate button to display mixture options.

5.4.10 All options that pass the heterozygous balance rule are displayed. Mx and MR are displayed for all calculated options. Use displayed tolerances to decide on options to include in any further evaluation/statistical calculations. **NB: These options are not ranked.**

LOCUS	Allele	Peak Height	Contributor 1		R	Contributor 2		Mx2	MR (x:1)	P	C	Notes
D19	12	600	12	12	↔	13	16	0.43	1.3	*	C	
	13	159	12	16	↔	12	13	0.37	1.7	*	C	
	16	300	12	16		13	F	0.15	5.7			

5.4.11 **P** column displays a preferred option (*****) based on Mx falling between tolerances.

5.4.12 Press **Mx Filter** to remove all options <u>not</u> falling between tolerances.

5.4.13 **IMB** will be displayed if there are detectable imbalances between alleles meaning no options are viable. Imbalance could imply that there may have been over peak adjustment, more than two contributors or peak imbalances.

5.4.14 **>2 Cont** indicates that due to the number of peaks there must be more than two contributors.

5.4.15 Press Locus buttons (e.g. [D8]) to display graphical representation of locus.
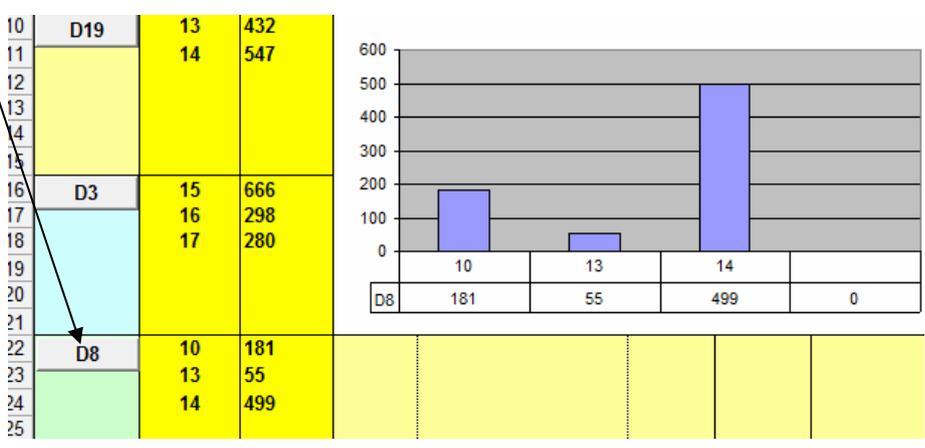
5.4.16 Put '*C*' on <u>any</u> options you would like to include in mixture calculations (LR, Match Probability) in the **C** column.

5.4.17 Print copy of mixture sheet for file.

5.4.18 Press button to make up calculation sheets

5.5 Calculation Sheets
5.5.1 The calculation sheets can be edited to remove options or add others.
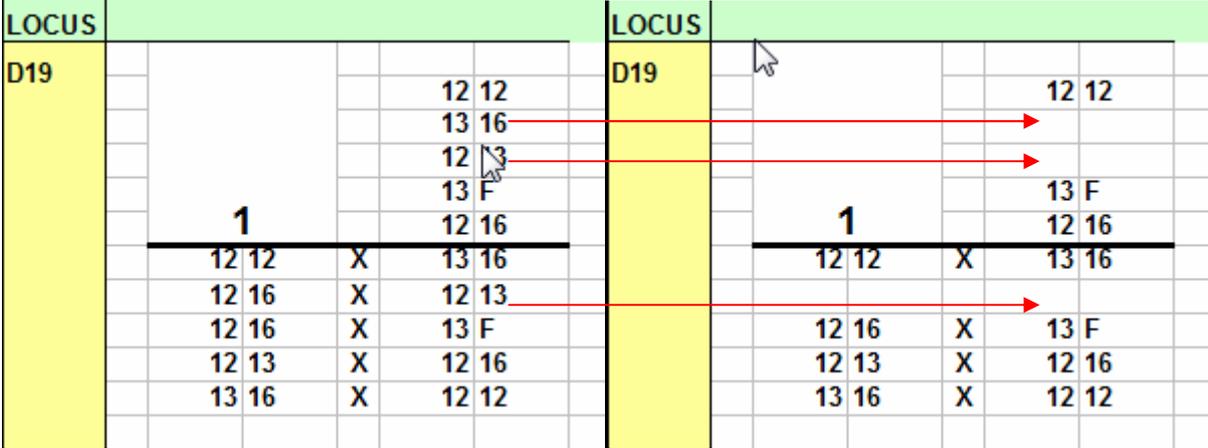
5.5.2 Editing the bottom line option in LRCalcCont 1 will automatically change them in LRCalcCont 2. Bottom line of LRCalcCont 2 cannot be edited.

5.5.3 Edit bottom line as program works out any reciprocal but doesn't filter for F

5.5.4 Edit and Print copies of calculation sheets for file.

5.6 Statistical Analysis

5.6.1 LR can be calculated for **Cont 1 + u/k** or **Cont 2 + u/k** via the buttons on either sheet. Go to "Caucasian Calculation" tab to print full LR paperwork if required.

5.6.2 Match probability and conditional match probability can be calculated via button prompts

5.6.3 Options can and may need to be edited - **NB: CHECK OPTIONS CAREFULLY**.
If there is swing between contributor 1 and contributor 2 of desired option program will compensate for this. Check options.

5.6.4 Match probability calculates "known over unknown" based on either Contributor Column.

5.6.5 Conditional Match Probability conditions on one of the contributors. **NB: CHECK OPTIONS CAREFULLY**. Conditional Match probability will calculate "Contributor 1 + Contributor 2 over Contributor 1 and Unknown" or "Contributor 1 + Contributor 2 over Contributor 2 and Unknown"

5.6.6 Print copy of Match Probability sheets for file.

Appendix 1- GeneMapper ID v2.3.1 Settings
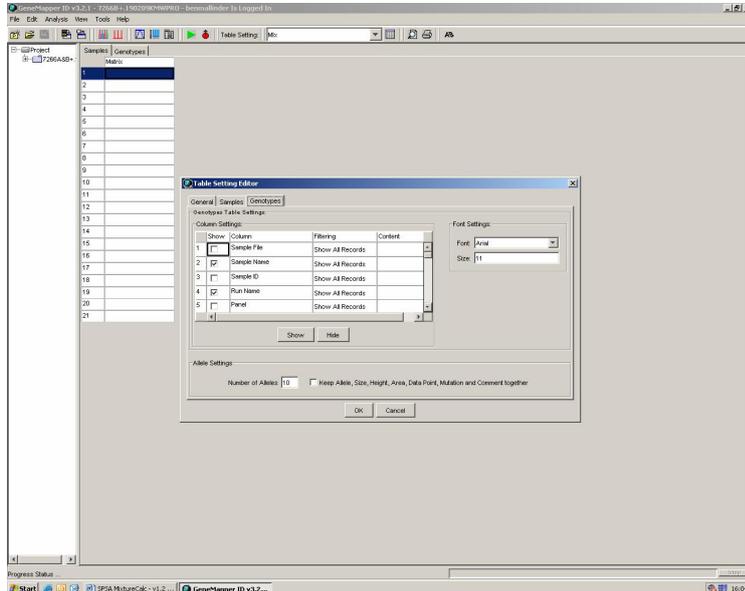
- Export analysed data to mixtures folder as .txt file

- GeneMapper export table should be set up as follows:

- In Table Setting Editor

- **General Tab** –   Table called "MIX"

- **Sample Tab** -   Column settings should have all boxes unchecked

- **Genotype Tab** –   Columns settings for Sample Name, Run Name, Marker, Allele and Height should be checked. All other boxes should be unchecked. No of Allele should = **10** Box to "Keep Allele, Size, Height, Area, Data Point, Mutation and Comment together" should be unchecked.