

Purpose:

MixMaster (Rel. 1.0).xlt was modified to create MixMaster IDP (Rel. 1.0).xlt

- Stochastic threshold (PHR range 2 start point) increased to 365 RFU
- PHR range 3 starts at 750 RFU
- PHR range 4 starts at 1200 RFU
- N+4 stutter threshold increased to 9.2% at all loci

Using Excel version 2000 and identical GMID export files, the Performance Check will compare the results obtained by...

- MixMaster (Rel. 1.0).xlt
- MixMaster IDP (Rel. 1.0).xlt

The focus of the performance check will be on those results that would be affected by the modifications.

Electronic Files used for the Performance Check:

- MixMaster spreadsheets tested
 - MixMaster (Rel. 1.0).xlt
 - MixMaster IDP (Rel. 1.0).xlt
 - Mx Assistant (BETA 1.4a).xlt
- Export txt files used for testing
 - mix_ID_3130_JB_KRD_M1F2 1ng_050511.txt
 - ref_ID_3130_JB_KRD_M_050511.txt

Note: These export files were previously used for the MixMaster performance check. The Genemapper allele calls for this mixture are identical using both the ID and ID Plus N+4 stutter thresholds.

Method:

1. Clicking on the "Import it!" button, I imported...
 - a. mix_ID_3130_JB_KRD_M1F2 1ng_050511.txt
 - i. See pages MMIDPV-1 to MMIDPV-2
 - ii. Note: For all tested MixMaster-Excel combinations, the software correctly applied the rules to guess at the name, but the casework naming conventions were not followed for this mixture study sample. The software predicted the name would be "1NG" based upon the full name fsa file name: "C04_BKCWMSI_1NG_2TO1_KRD_003.2.fsa". I modified this name to "1NG 2 to 1".
 - b. ref_ID_3130_JB_KRD_M_050511.txt
 - i. See pages MMIDPV-3
 - ii. Note: For all tested MixMaster-Excel combinations, the software correctly applied the rules to guess at the name, but the casework naming conventions were not followed for this mixture study sample. The software predicted the name would be "XXCWP2" based upon the full name fsa file name: "F02_KRD_XXCWP2_H-19a1_002.2.fsa". I modified this name to "XXCWP2 M".
2. Clicked on the "Run it!" button.
3. Upon completion, saved the files:
 - a. MixMaster (Rel. 1.0) Excel 2000 Rapid.xls

*JWL - reviewed
APP 1-3, MMIDPV 1-7
MMIDPV ARCHIVE 1
11/7/12*

*T/L Ramey
12/21/12
mm*

- b. MixMaster IDP (Rel. 1.0) Excel 2000 Rapid.xls
4. Using the drop-down menus and the "Report it!" button, I created reports for the combinations Not Reduced and No Assumed. This allowed me to compare all of the allowed genotype pairs.
5. Clicking on the "Print it!" button, I printed the four reports (I had set the printer to PDF for this performance check.) Saved the PDFs, where "[Run file name]" is the respective file name from step 3 (above): [Run file name] Not Filtered-Not Reduced.pdf
 - a. See pages MMIDPV-4 to MMIDPV-7

Results:

1. Data entry, Mx calculation and range, run times, and report creation were consistent and functional across the two versions.
2. All genotype pairs interpreted using MixMaster (Rel. 1.0).xlt were included in the results of MixMaster IDP (Rel. 1.0).xlt.
3. MixMaster IDP (Rel. 1.0).xlt included additional interpreted genotype pairs. The causes for the differences were examined using Mx Assistant (BETA 1.4a).xlt and the relevant kit/protocol settings.
 - a. D8S1179
 - i. 14,F minor + 10,15 major: $\geq 10.7\%$ N-4 stutter from the 15 allele brings the height of the 14 allele below the IDP stochastic threshold (365 RFU.) Max N-4 stutter from the 15 allele still leaves the 14 allele above the ID stochastic threshold (200 RFU.)
 - b. D21S11
 - i. 29,F minor + 30,30 major: $\geq 3.5\%$ N-4 stutter from the 30 allele brings the height of the 29 allele below the IDP stochastic threshold. Max N-4 stutter from the 30 allele still leaves the 29 allele above the ID stochastic threshold.
 - c. CSF1PO
 - i. 12,F minor + 10,11 major: The 12 allele is below the IDP 365 RFU stochastic threshold but above the ID 200 RFU stochastic threshold, even after the maximum 4.5% N+4 stutter from the 11 allele is subtracted from the 12 allele
 - d. D13S317
 - i. 11,12 minor + 12,13 major: Using the ID settings, reducing the height of the 13 allele by subtracting 4.5% N+4 stutter due to the 12 allele, and reducing the height of the 11 allele by subtracting 11% (max) N-4 stutter due to the 12 allele, the 12 allele could never be split in such a way that both contributors would have an acceptable ID PHR. Using the IDP settings, there are multiple ways to make this combination work. You could skip stutter subtraction and just adjust the allele sharing (one split will give both donors an acceptable PHR of 40.37%.) Or you could leave the 12 evenly split but subtract from the 13 allele $\geq 7.1\%$ N+4 stutter due to the 12 allele.
 - ii. 12,13 minor + 11,12 major: See above. The Mx is OK with either as the major.

e. D19S433

- i. 11.2,13 minor + 13,14 major: Using the ID settings and reducing the height of the 14 allele by subtracting 4.5% N+4 stutter due to the 13 allele, the 13 allele could never be split in such a way that both contributors would have an acceptable ID PHR. Using the IDP settings and reducing the height of the 14 allele by subtracting ~8.6% N+4 stutter due to the 13 allele, you could adjust the allele sharing to give both donors an acceptable PHR of ~30.5%.
- ii. 11.2,14 minor + 11.2,13 major: Using the ID settings and reducing the height of the 14 allele by subtracting 4.5% N+4 stutter due to the 13 allele, the 11.2 allele could never be split in such a way that both contributors would have an acceptable ID PHR and still be within the Mx range. Using the IDP settings and reducing the height of the 14 allele by subtracting 9.2% N+4 stutter due to the 13 allele, you could adjust the allele sharing to give both donors an acceptable PHR of 33% for the "minor" (taller allele ~723 RFU) and 67.87% for the "major" (taller allele 372 RFU.)
- iii. 13,F minor + 11.2,14 major: Subtracting from the 13 allele even ~1% N-4 due to the 14 allele brings the 13 allele below the IDP Stochastic threshold.. Subtracting maximum N-4 stutter still leaves the 13 allele above the ID Stochastic threshold.

f. D18S51

- i. 12,14 minor + 17,22 major: Using the ID settings, this combination is eliminated based upon PHRs. Even subtracting from the 17 allele maximum (4.5%) N+4 stutter due to the 16 (stutter-filtered) peak, the PHR of the major donor is still outside the ID ranges. Using the IDP settings, this combination is within PHR range regardless of stutter subtraction.
- ii. 17,22 minor + 12,14 major: See above. The Mx is OK with either as the major.

g. D5S818

- i. 12,F minor + 9,11 major: ^{N+4} Max N+4 stutter due to the 11 allele still leaves the 12 allele above the ID 200 RFU stochastic threshold. Without any stutter subtraction, the 12 allele is already below the IDP stochastic threshold.
- ii. 8,12 minor + 9,11 major: Using the ID settings, this combination is eliminated based upon PHRs. Even subtracting from the 12 allele maximum (4.5%) N+4 stutter due to the 11 stutter-filtered peak, the PHR of the minor donor 12 allele with the stutter-filtered 8 peak is still outside the ID ranges. Using the IDP settings, this combination is within PHR range regardless of stutter subtraction.

Conclusion:

The two versions of MixMaster gave concordant results except for differences that were expected given the Stochastic, PHR, and N+4 stutter threshold changes.

