Intermountain Forensics	SOP #	IAC-200
	Revision #	04
Forensic DNA Technical Leader Approval	**Issue Date**	
signature	03/01/2023	

ArmedXpert™ and MixtureAce

1. Purpose

To provide instructions for the ArmedXpert™ analysis software and MixtureAce plugin.

2. Summary

ArmedXpert™ provides mixture interpretation assistance by providing calculations based on analyst input parameters. Statistical calculations are also provided based on the input of the analyst's interpretations. Allele Summary Tables and Allele Bar Charts can be printed to be added into case files. The MixtureAce plugin allows upload of FastQ files for interpretation of NGS generated data.

3. Procedure

ArmedXpert™ Log In
1. Open the ArmedXpert™ software for the desired amplification kit.

2. Click **OK**.

Import Files for CE:

1. Click the icon in the upper left corner.

2. Hover over **Import**, then **click on Other**.

3. Navigate to the case folder and open the Genotype table.

Import Files for NGS into MixtureAce:

1. Click the icon in the upper left corner.

2. Hover over **Import**, then **Plugins**, and click on the MixtureAce plugin.

3. Browse and select the desired FastQ files and kit(s).
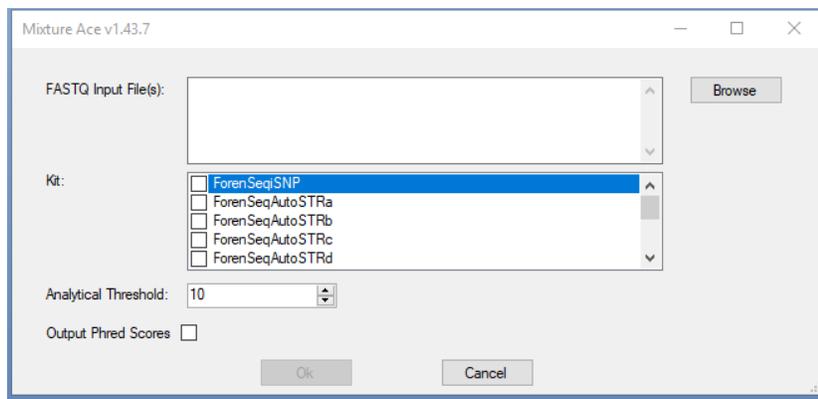
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      a. AutoSTR (a,b,c,d)

            i. STR kit "d" matches the NIST database and should be used for all statistical comparisons.

      b. YSTR(a,b,c)

      c. XSTR(a,b,c)

      d. iSNP

4. When multiple FastQ files are imported, separate project files(.axn) are created automatically.

    1. Sample Projects may be interpreted individually or combined for cases with multiple items.

Combine Project Files:

1. Add all of the desired .axn files into the same folder.

    a. The Merge MixtureAce Projects by Kit Plugin will combine all .axn files in the folder.

2. Click the icon in the upper left corner.

3. Hover over **Import**, then **Plugins**, and click on the Merge MixtureAce Projects By Kit button.

4. Find the desired project file folder.

5. Click on any (.axp) project in the folder and click Open.

Import Project files:

1. Click the icon in the upper left corner.

2. Hover over **Import**, then **Project**, and click on the Project button.

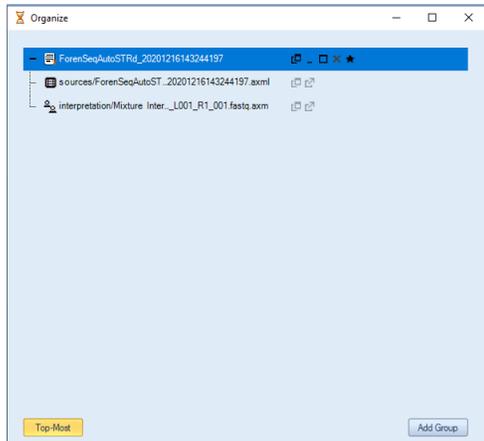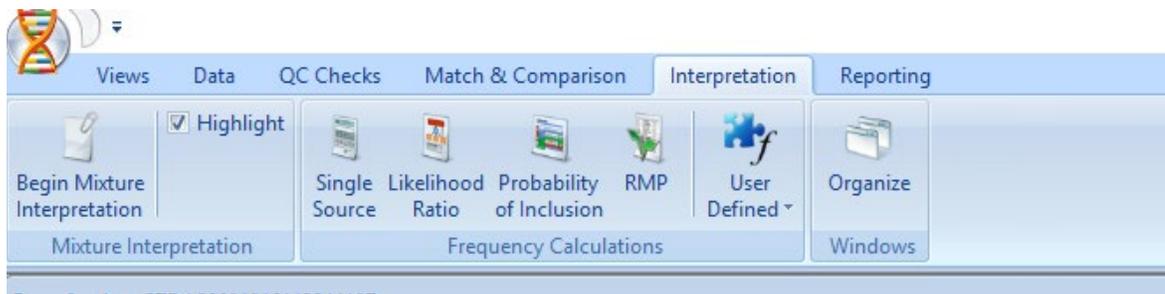|	**Intermountain Forensics**	SOP #	IAC-200
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3. Find the desired project file (.axp) and click Open.

4. Click on the + to dropdown the project file.

5. Double click on the project or interpretation file.

Sample Interpretation:

6. Click on the Interpretation tab.

   1. Shortcut is holding Alt and clicking on the sample name or any of the alleles.

   NOTE: Do not close out of the Mixture Interpretation unless you have exported/saved or printed the reports you require to document your interpretation. If not, once you close out of the Mixture Interpretation tool all information will be lost.

7. Click **Begin Mixture Interpretation** and select sample through either Pick via mouse or selecting in the dropdown.

   1. Holding Alt and selecting the sample name will also open the Mixture Interpretation window.

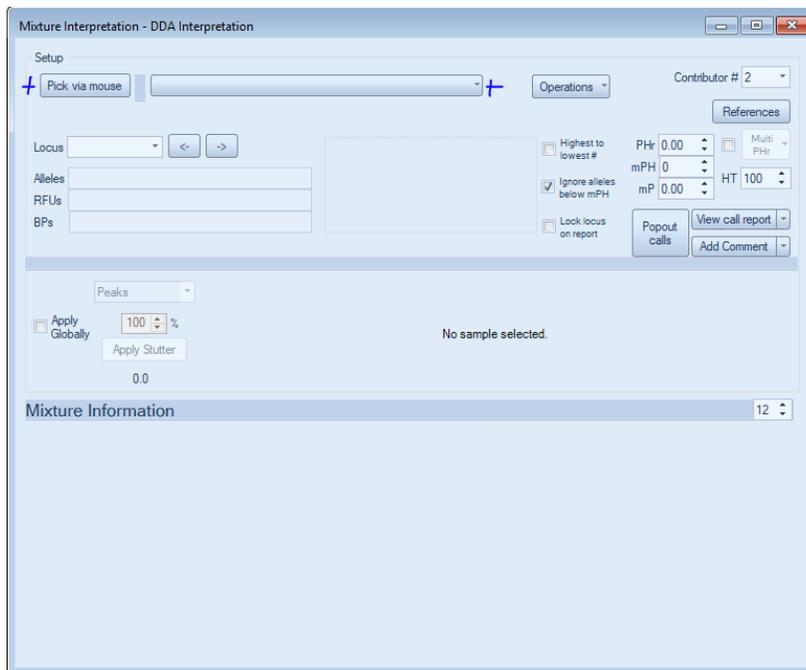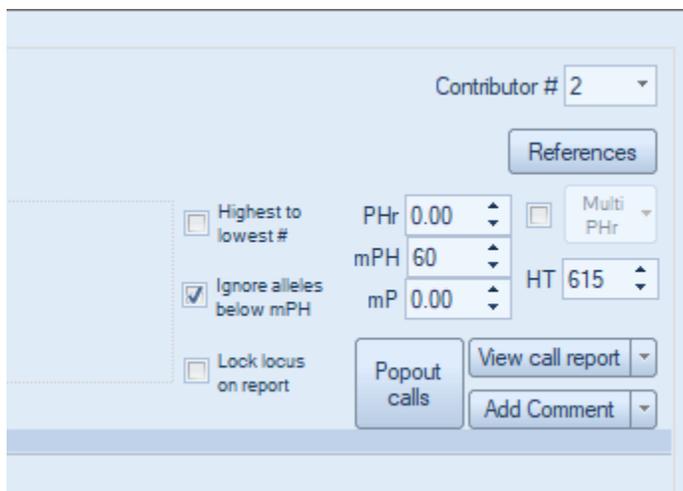|	Intermountain Forensics	SOP #	IAC-200
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8. Enter the number of contributors and ensure that the Highest to lowest # is checked.

    a. This assists in providing more accurate contributor ratios for the loci with fewer called alleles.

9. If the sample has any assumed contributors, click the **References** button to apply the reference(s).

10. Click **Select a reference to apply.**

    a.   Clicking on **Auto-call references** will automatically fill in as the first profiles.

11. Modify the profile name with the Item identifier (IMF-XX-XXXX-XX).
    1.   Unknown Profile/Haplotype 01, Unknown Profile/Haplotype 02, Unknown Profile/Haplotype 03
    2.   Mixture
    3.   Co-Major

12. Contributor possibilities will populate depending on the interpretation settings and number of contributors selected.

13. PHr may be adjusted based on analyst discretion and the new calculations will populate.

14. Assign contributor alleles in one of three ways.
    a. Right click the mixture combination and send to desired profile.

15. Check alleles from the middle section.

16. Clicking on **Popout calls** will open the allele data in a new window.

17. Checkmark assigns allele.
    a. No dropdown- Assigns the allele as a possibility
        i. Use when assigning completely typed locus
    b. Allele name- Allele possible, no dropout
    c. Any- Dropout possible
    d. Allele name*- Obligate
    e. Ignore Allele- Allele not used
    f. Remove Allele- Completely deletes the allele

g. [Peak] Make stutter- Allele not used
18. Assignment on the statistics page
    a. More than 2 possible alleles-No dropout
    b. Click dropdown and select "All Hets"
    c. More than 2 possible alleles-all under the stochastic threshold
    d. Click dropdown and select "Forced mRMP"
19. Make decisions on each locus based on the peak height ratio calculation and the mixture proportions.
    a. Change loci using the arrow buttons

20. The Contributor contribution updates as decisions are made.
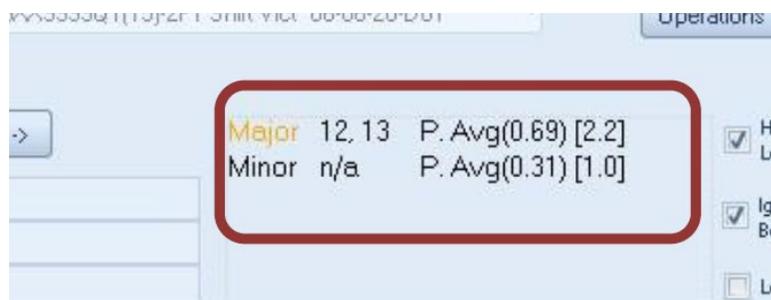
21. Locus ratios can be visualized for comparison by clicking View call report, clicking on the Profile Proportions tab and scrolling down to the bar chart.
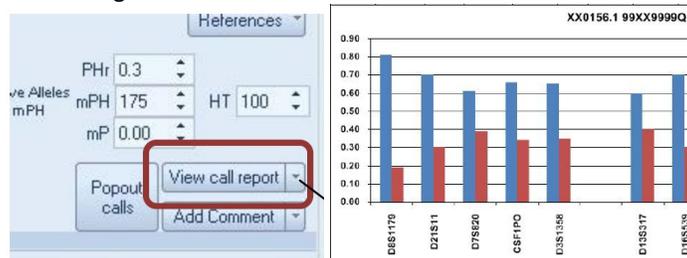
Stutter Assessment (MixtureAce)
    1. Opening the Allele Bar Chart (ABC) and hovering over the alleles will show the repeat motif.
    2. The same motif indicates that consecutive alleles are in stutter position.
    3. Clicking the **Apply Stutter** button removes the max stutter percentage from allele read counts in stutter positions.

Sequencing Errors (MixtureAce)
    1. Check the NIST database to determine if it is a previously reported allele
    2. Find where the sequence difference is in either the UAS Genotype Report or Flanking Report.

Opening a saved interpretation
    1. Click on Organize in the Interpretation tab.

2. Open the project options if necessary.
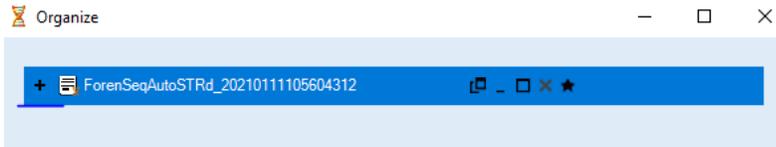
3. Double Click on the desired sample interpretation

Statistics
1. Statistics are generated on allele frequencies obtained from the NIST 1036 Revised U.S. Population Dataset, published in July 2017.
    a. Minimum Allele Frequency: The frequency of 5/2n was used for any allele that was observed less than five times in the population database.
2. Single source sample statistics
    a. Click RMP under the Interpretation tab.
    b. Hold Alt and select the sample name.
    c. Verify all loci have the intended statistics and modify as necessary.
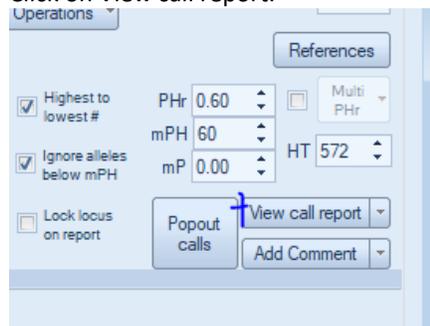   d. Change the number of contributors if necessary.
    e. Print by clicking on the icon and clicking Print and Print in the next tab.
    f. Click Fit to pages and change "tall" to 2 or 3.
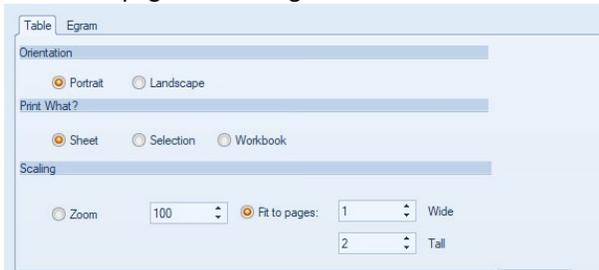
    g. Click Print and save.

3. Mixtures Statistics
    a. Open the sample interpretation window if necessary (25a-c)
    b. Click on View call report.

    c. Click RMP under the Interpretation tab.
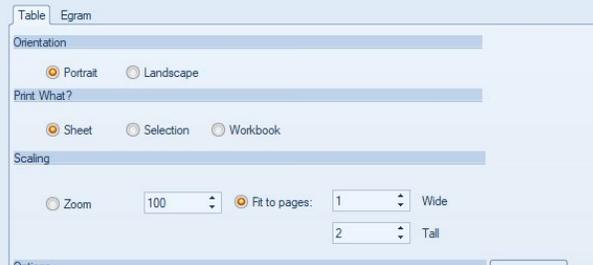
    d.   Hold Alt and select the sample name or individual name.

    e.   Print by clicking on the icon and clicking Print and Print in the next tab.

    f.   Click Fit to pages and change "tall" to allow the pages to be readable.

    g.   Check in Print Preview

    h.   Depending on the mixture, the page layout may need to be changed to Landscape.

    i.   Click Print and save

Generating an Allele Summary Table (AST)
1. With the project screen as the last window clicked:

    1.   Click on the icon, hover over Export, Plugins, and click on Vertical Report without RFUs.
    2.   Verify the allele calls in the Allele Bar Chart are consistent with the allele calls from the electropherogram, including stutter and artifact calls, prior to adding the document to the support documents file.

2. To add sample contributor profiles:
    1.   Open **View call report**

    2.   Click on the icon, hover over Export, Plugins, and click on Vertical Report without RFUs
    3.   Copy and Paste sample contributor profiles into sample AST.

Generating an Allele Bar Chart (MixtureAce)
1. Either Click on the **Operations** Drop-down menu in the Mixture Analysis window and select **Open OSIRIS Plot File** or hold Ctrl + Alt and select the item.
2. Alleles may be modified as stutter or artifact by clicking on the allele, selecting artifact or stutter and clicking **Relabel**.

3. Print by clicking on the icon and clicking Print and Print in the next tab.
4. Verify the allele calls in the Allele Bar Chart are consistent with the allele calls from the electropherogram, including stutter and artifact calls, prior to adding the document to the support documents file.

4. References

ArmedXpert™ User's Manual

5. Definitions

N/A