SOFTWARE RELEASE NOTES

Converge™ Software 2.2
NGS Data Analysis 1.2

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PRODUCTS AFFECTED

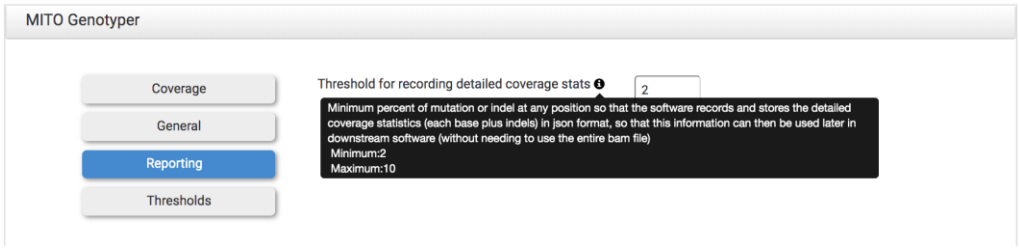
- Converge Software v2.2
  - HID Genotyper Plugin v2.2
  - Next-Generation Sequencing Module for STR, SNPs and Mito - Converge-NGS-1.2.zip
  - Upgrade Installer Files – upgradecvg (v1.0)

SOFTWARE OVERVIEW

Converge is a multi-phased product suite for Next Generation Software Platform offering upstream data storage and workflow capabilities, genotype calling and tertiary analysis tools. The software supports NGS STR, mtDNA and SNP (ancestry and identity) data analysis as well as Kinship and Paternity testing for global HID customers.

KEY FEATURES IN CONVERGE v2.2

- Mito IGV Lite: Converge user interface now includes a web-based compressed view of IGV read pileup on Mito results page to view the coverage for variants directly in Converge.
  - User can define the % difference value (2% - 10%) to determine which base position data is displayed in the user interface. Default value is 2%.
  - Any base position with higher percentage than the value assigned will be shown in IGV Lite.

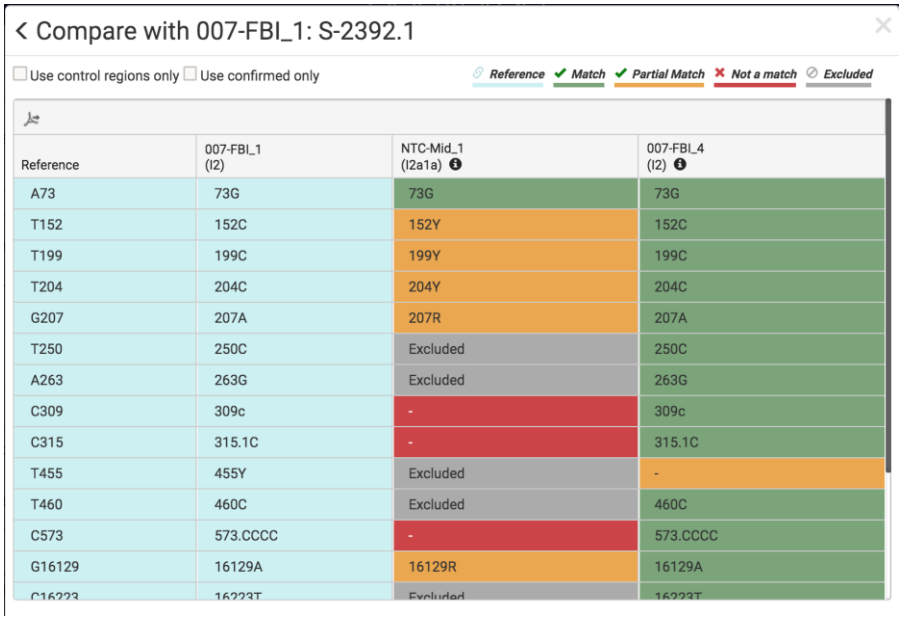


- Mito TS plugin output folder : A link from results page that navigates to the Torrent Server folder containing raw files associated with the particular sample.

- Mito View and search haplogroup : Users can view and search for mtDNA haplogroups.

- Haplogroup information is visible on the profile details page.
- Haplogroups can be searched on the profile dashboard.

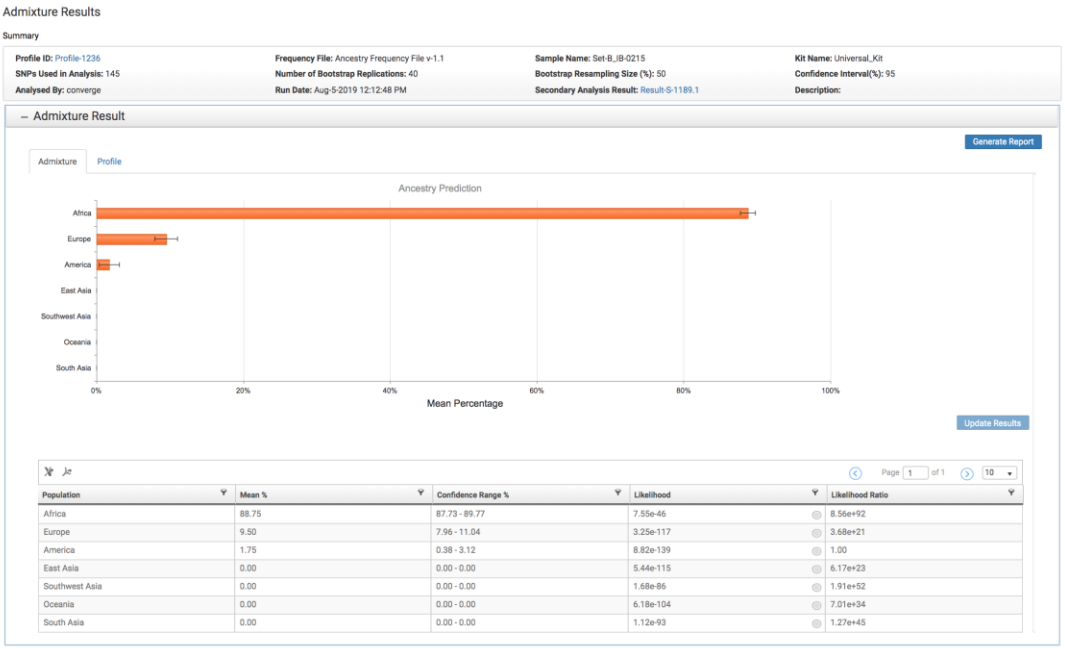
- Mito CODIS export: mtDNA profiles can be exported the CODIS .cmf v4.1 file format.
  - Originating Agency Identifier (ORI) was added in Organizational Details screen.
  - Mito CODIS exports will include the ORI number from the Organizational Details.
- Mito profile comparison: If a user performs profile comparison with a sample(s) containing point heteroplasmy (PHP), IUPAC codes are used, and the PHP variants are highlighted in orange.
  - Reference column format is #BASE#POSITION
  - For whole genome sample comparison, the option "Use control region only" is enabled if a user wants to restrict the comparison to only the control regions.



Reference	007-FBI_1 (I2)	NTC-Mid_1 (I2a1a)	007-FBI_4 (I2)
A73	73G	73G	73G
T152	152C	152Y	152C
T199	199C	199Y	199C
T204	204C	204Y	204C
G207	207A	207R	207A
T250	250C	Excluded	250C
A263	263G	Excluded	263G
C309	309c	-	309c
C315	315.1C	-	315.1C
T455	455Y	Excluded	-
T460	460C	Excluded	460C
C573	573.CCCC	-	573.CCCC
G16129	16129A	16129R	16129A
C16223	16223T	Excluded	16223T

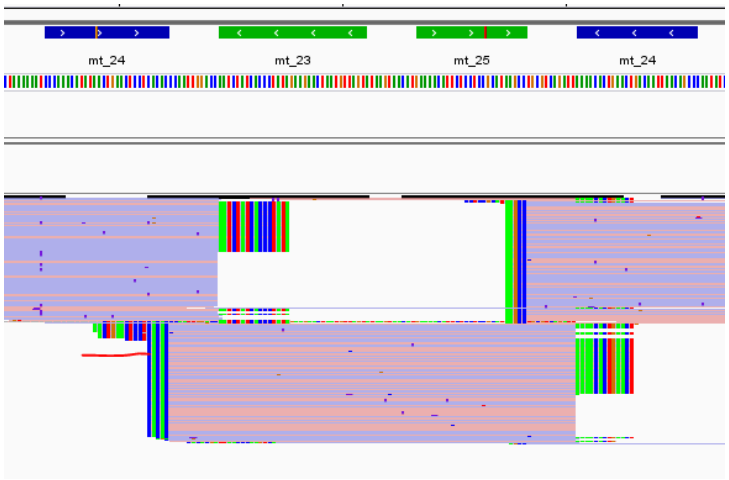
- SNP Ancestry Prediction: Improved ancestry predictions performed by bootstrapping the admixture estimations, and the variability between predictions is used to obtain a likely range in estimates using a user-defined sampling percentage and number of bootstrap replications. In samples where there is uncertainty due to less distinction between the constituent population groups, the results should show larger confidence interval around the predicted means.
  - References:
    - Kidd et. al. Poster: Better SNPs for Better Forensics: Ancestry, Phenotype, and Family Identification. Shown at National Institute of Justice annual meeting, Arlington VA, June 2012.
    - Kosoy R, Nassir R, Tian C, et al. (2009) Ancestry informative marker sets for determining continental origin and admixture proportions in common populations in America. Hum Mutat 30(1) 69–78.
  - In the analysis settings, users select an Ancestry Frequency File v1.1 to display the number of SNPs in the panel and designate (a) Number of Bootstrap Replications, (b) Bootstrap Resampling Size (%) and (c) File Description to perform this tertiary analysis.
  - Note:
    - Admixture analysis between 2.1 and 2.2 are not compatible; any 2.1 admixture analyses should be downloaded before upgrading the software.
    - Profiles generated in 2.1 will remain intact following software upgrade. Users can re-analyze the profiles with the new bootstrap admixture analysis in 2.2.

- The Admixture Results displays the following information:
  - Ancestry composition shown as a mean of ethnicity percentages and confidence intervals
  - Probable range of variability of the estimated ethnicity percentages
  - Confidence level of the variability estimate in a percentage
  - Bootstrapping sampling percentage and number of samplings



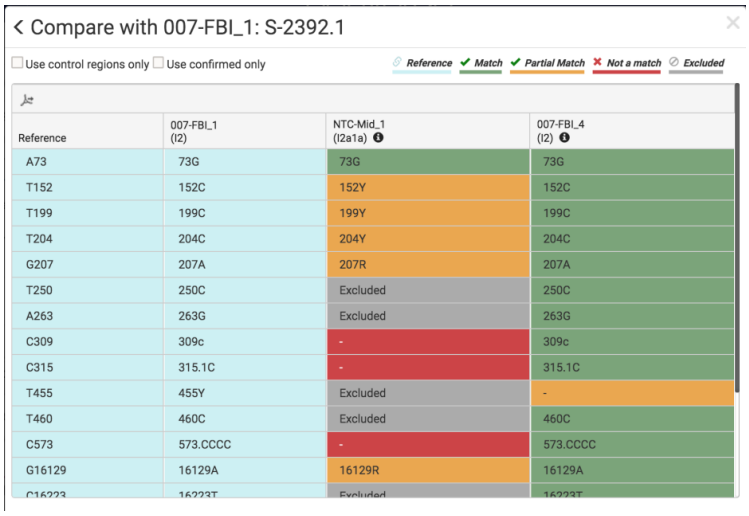
- Custom SNP panel workflow: The software is now enabled to perform custom SNP genotyping and analysis. This feature allows customers to perform the following options with a custom SNP panel designed by the user:
  - Create, upload and analyze the panel
  - Custom SNP haplotyping and genotyping
  - Ancestry and identity analysis based off user supplied populations and allele frequency data
  - Custom admixture analysis
- Bulk association of samples : User can link more than 1 sample at a time to a case in sample dashboard and on NGS Batch page.

- Bug fixes:
  - MtDNA primer sequences including fusion products are now removed bringing significant improvement in MVC analysis with fewer false positives which is most evident in NTC and samples with low coverage.



- MtDNA Consensus fasta file now has as many contigs as there are regions (3 for 3 regions, 5 for 5 regions etc).

- IUPAC codes are now used in Converge UI for profile comparison. Profile comparison now also includes regions analyzed; non overlapping regions are greyed out.



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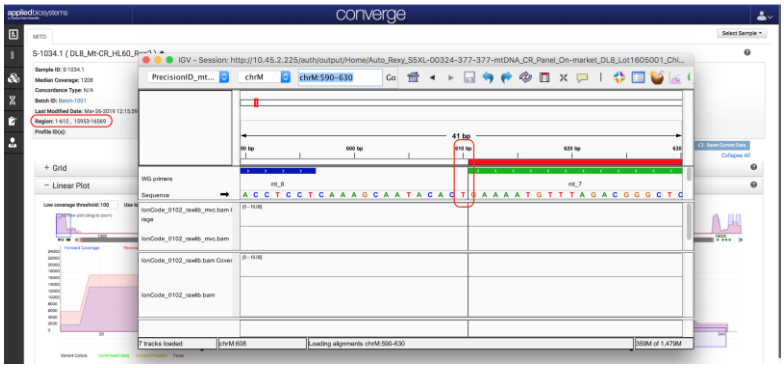
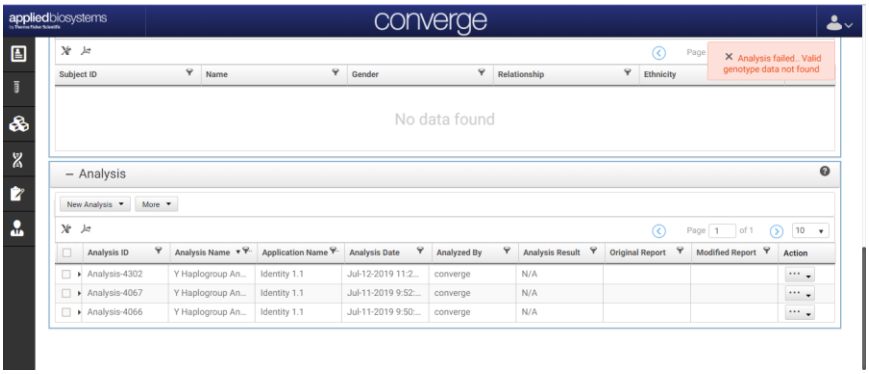
T250	250C	Excluded	250C
A263	263G	Excluded	263G
C309	309c	-	309c
C315	315.1C	-	315.1C
T455	455Y	Excluded	-
T460	460C	Excluded	460C
C573	573.CCCC	-	573.CCCC
G16129	16129A	16129R	16129A
C16223	16223T	Excluded	16223T

- In v2.1, excluded regions in Mito IGV were offset by 1 base; this issue is now resolved.



- For Y Haplogroup analysis, "Valid genotype data not found" is now displayed when all markers have genotype as "N" for female profiles.



SYSTEM REQUIREMENTS CONVERGE 2.2:

- TSS v5.10 / Ion GeneStudio S5, Ion Gene Studio Plus, Ion GeneStudio Prime/ Ion S5XL™
- Converge Software Server & its components
  - Dell™ PowerEdge™ T130 Tower Server, motherboard v2 or later
  - Red Hat™ Enterprise Linux™ operating system
  - Apache™ Tomcat™ application server that runs on Converge software
  - PostgreSQL database server that stores the data for the server and software
  - Google™ Chrome™ browser
  - Automatic configuration of IP, domain name service (DNS), and Windows internet name service (WINS) settings via dynamic host configuration protocol (DHCP)
- Converge Software Server Specifications
  - Processor - Intel™ Xeon™ Processor E3-1270 v6, 3.8 GHz, 8M cache, 4C/8T,turbo (72 W)
  - Memory - 16 GB of memory (2 × 8 GB), UDIMM, 2400 MT/s, Single Rank, x8 Data Width, DVD ROM, SATA, Internal
  - Hard Drive (2) - 2 TB 7.2 K RPM NLSAS, 12 GB/s, 3.5-in cabled hard drive (RAID1)
  - Data Storage - RAID 1; PERC H330 Integrated Controller for 3.5-inch cabled hard drive
  - Operating System - Red Hat™ Enterprise Linux™ operating system
  - Browser - Google Chrome™ 66 or later
- Recommended Software (not provided)
  - Adobe™ Acrobat Reader
  - Microsoft Excel
- Verified Converge 2.2 ™ software workflow on Google Chrome™ and MAC Safari browsers.

INSTALLATION / UPGRADE:

SOFTWARE RELEASE NOTES

Refer to Converge™ Software v2.2 SETUP AND REFERENCE GUIDE - Publication Number 100039539, Rev E for following instructions

- Initial setup and configuration of Converge™ Software Server and Converge™ Software.
- Managing the Converge™ Software Server and licenses.
- In addition, following sections covers Upgrade and Fresh Install workflow plus enhanced troubleshooting section.
  - Appendix A - Troubleshooting Server networking, Password Issues, Access to log files, Restart Services, Reset IP address, Account Configuration and Dell T110 USB recognition.
  - Appendix B - Upgrade to Converge 2.1 and Converge 2.2 on Dell T110 and T130 Appliance Servers.

Publication Number 100039539, Rev E can be found here.

Steps below provide additional reference links for TSS upgrade to 5.10 and supplemental files that need to be downloaded prior to starting an end to end run from TSS and generating a batch file on Converge™ Software.

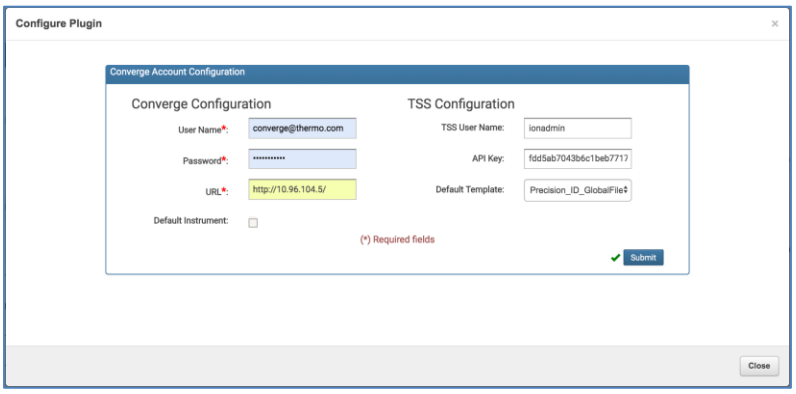
- TSS - Upgrade your Torrent Server, Ion Chef, and Ion S5/S5 XL to TSS v5.10. For TSS v5.10 documentation, refer to the TSS 5.10 User Guide and Release Notes.

- Precision_ID_Panel_Definitions.zip - Includes relevant Reference/BED and JSON Files and must be installed onto TSS v5.10 before analyzing data generated with Precision ID Chemistry. The zip file contains the following list of files and can be downloaded from S3 link.

  List of files include (*Note: These files have not changed since Converge 2.1*)

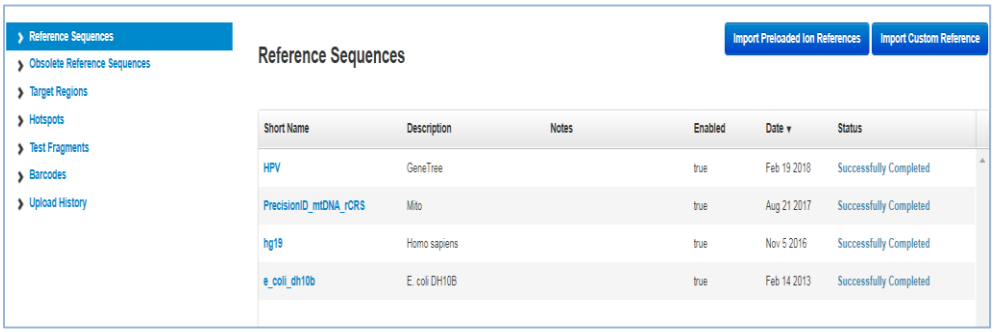
  a. For Mito Panel –
     - Mito Reference - Precision_ID_mtDNA_rCRS
     - Mito CR Target File - Precision_ID_mtDNA_Control_Region_Panel_Targets_v1.0.bed
     - Mito WG Target File - Precision_ID_mtDNA_Whole_Genome_Panel_Targets_v1.0.bed
     - Mito Analysis Parameter File - Precision_ID_mtDNA_Panel_AnalysisParams_v1.0.json
  b. Analysis Parameter File - Precision_ID_mtDNA_Panel_AnalysisParams_v1.0.jsonP Panel
     - Ancestry Target File - Precision_ID_Ancestry_Panel_Targets_v1.0.bed
     - Ancestry Hotspot File - Precision_ID_Ancestry_Panel_Hotspot_v1.0.bed
     - Ancestry Analysis Parameter File -Precision_ID_Ancestry_Panel_AnalysisParams_v1.0.json
     - Identity Target File - Precision_ID_Identity_Panel_Targets_v1.0.bed
     - Identify Hotspot File - Precision_ID_Identity_Panel_Hotspot_v1.0.bed
     - Identity Analysis Parameter File - Precision_ID_Identity_Panel_AnalysisParams_v1.0.json
  c. STR Panel
     - Precision_ID_GlobalFiler_NGS_STR_Panel_Target_v1.1.bed
     - Precision_ID_GlobalFiler_NGS_STR_Panel_Hotspot_v1.1.bed
     - Precision_ID_GlobalFiler_NGS_STR_Panel_AnalysisParams_v1.1.json
     - Precision_ID_GlobalFiler_NGS_STR_Control_Sample_male007_v1.1.json
     - Precision_ID_GlobalFiler_NGS_STR_Control_Sample_9947A_v1.1.json
     - Precision_ID_GlobalFiler_NGS_STR_Control_Sample_NegCtrl_v1.1.json
     - Precision_ID_GlobalFiler_NGS_STR_Control_Sample_9947A_and_male007_and_NegCtrl_v1.1.json

- HID Genotyper v2.2 Plugin - Download the plugin HIDGenotyper-2.2.zip from S3 link or installation and upload / configure plugin on supported S5/S5XL ™ Torrent Suite server. An example of successful configuration of HID Genotyper v2.2 Plugin with TSS and Converge v2.2 software is shown below



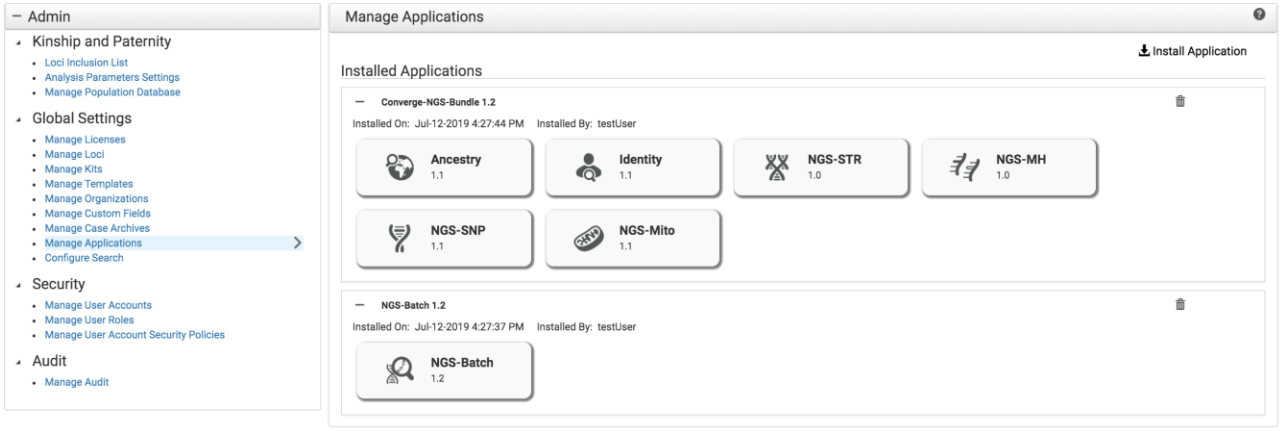
- Upgrade Installer Package – Download following two files (Serial Number - 4b0cd383b-201908300532) from S3 link for upgrading Converge v2.1 to Converge v2.2 software installed on Dell T110 or T130 Server.
  Refer to Converge™ Software v2.2 SETUP AND REFERENCE GUIDE - Publication Number 100039539, Rev E (Appendix B) for instructions on upgrading to Converge v2.2 software
  - ConvergeUpgrade-2.2-0.x86_64.rpm - S3 link
  - upgradecvg - S3 link

- Download Precision_ID_mtDNA_rCRS File (NCBI reference NC_012920) file from S3 link and upload onto TSS > References page (snapshot below)



- Readme.txt instructions can be downloaded from S3 link to a USB and inserted into a readable port of the Converge appliance server for use. For any technical support on upgrade path, contact local FAS team member.

- Converge-NGS-1.2.zip - Download Converge-NGS-1.2.zip file from S3 link.
- Post upgrade from Converge v2.1 > Converge 2.2 version, all licenses will be intact, and user need not install it again since its and upgrade from 2v.1, followed by uploading NGS Application Bundle package onto Converge > Admin > Manage Application Software page (snapshot shown below).



NOTE:

- If earlier version of the Converge ™ Software included the Kinship and Paternity module and/or the NGS module, and the licenses have not expired, the licenses are retained after the upgrade. Licenses need not be reinstalled.

UPDATES TO CONVERGE™ SOFTWARE v2.2 HELP TOPICS

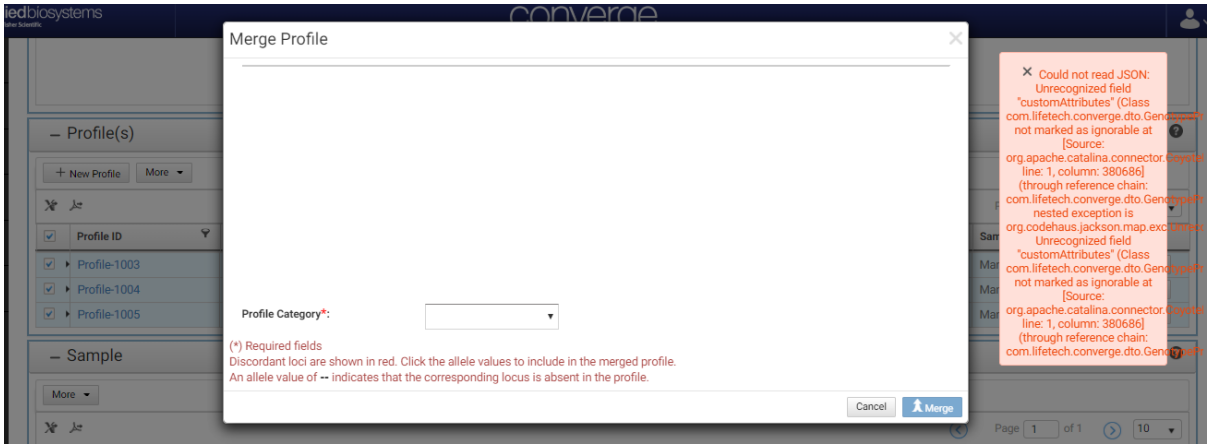
Refer to Converge™ Software v2.2 help topics within the application for more information on the following updates:

- Added the following new features and updated procedures per changes to the UI workflow.
  - Updated Ancestry algorithm workflow
  - Bulk association of samples to a case
  - Mito IGV light
  - Profile comparison legend
  - Inclusion of Mito regions information in Profile
  - View and search Mito profiles based on Haplogroups
  - New "Reporting" parameter for Mito in HID Genotyper plugin
  - Uploading and analyzing custom panels
- Troubleshooting section has been enhanced for following sections and recommended actions discussed.
  - NGS Module workflow, Display Issues, Profile Management, and Kinship & Paternity features.

KNOWN ISSUES AND LIMITATIONS:

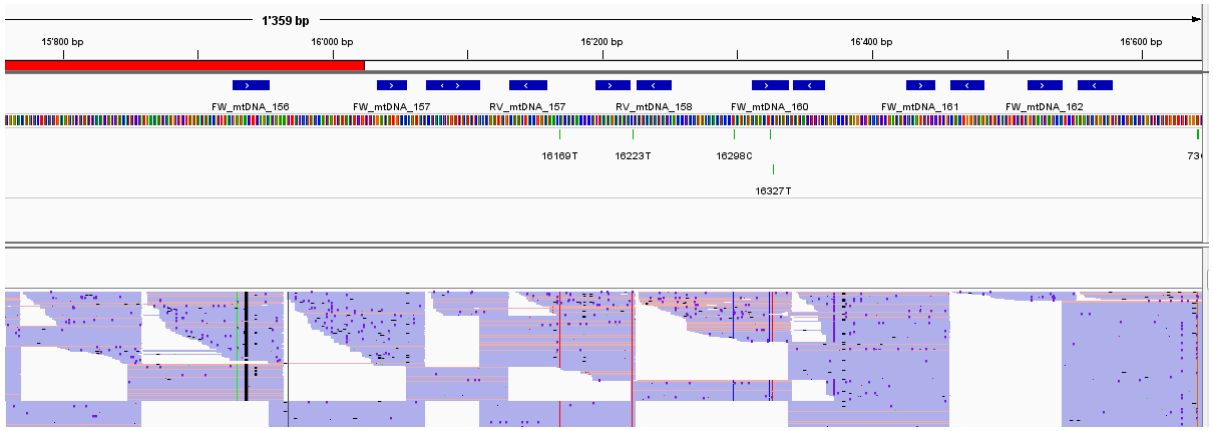
- **General functionality related:**

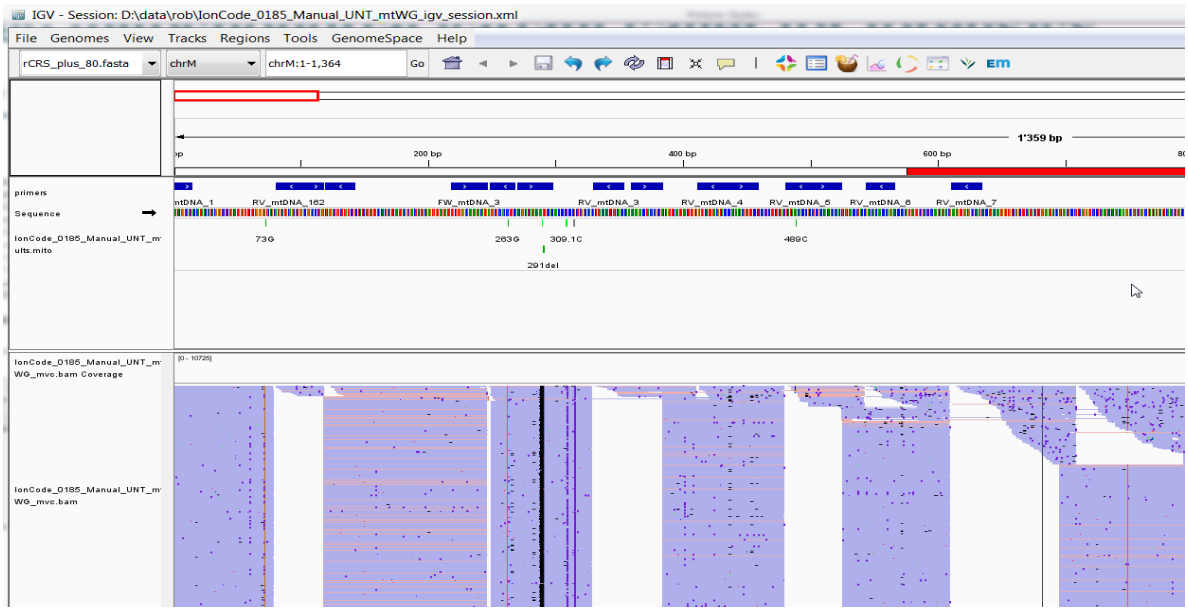
  - The profile merge feature will not allow two mtDNA profiles to be combined when selected on Case Details Page > Profile card. See attached screenshot for error message.



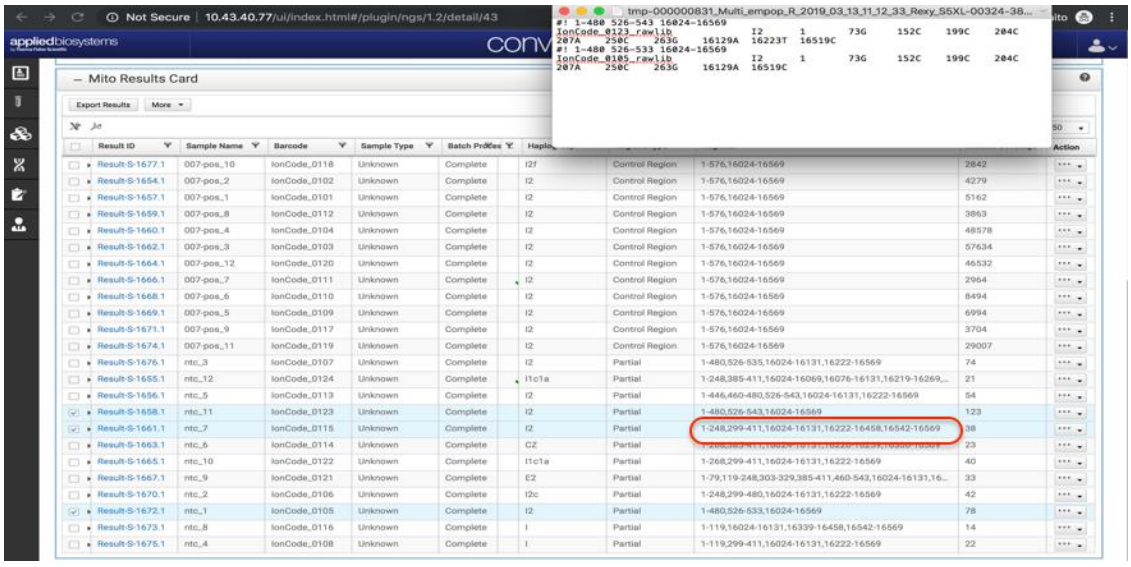
- The Control Region panel spans the region from 15,954 – 610. Converge will display only the Control Region sequence (16,026 – 576) when that analysis display is chosen. If users want to view the full sequence, the data must be viewed in IGV by accessing the data from Torrent Server.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V
1	Date	2018-08-08																				
2	Bam file	D:/data/rob/IonCode_0185_Manual_UNT_mtWG_results/IonCode_0185_Manual_UNT_mtWG_mvc.bam																				
3	Sample	Samp	MQKYQ.IonCode_0185																			
4	Close hapl	C1																				
5	Regions	1-574 16024-16569																				
6																						
7	Position	Ref	Samp	Varia	Var Freq	Type	Read Cove	Read Cove	Allele Cov	Allele Cov	Allele G%	A%	T%	C%	N%	ins%	del%	Polymorp	Control Re	State	Freque	
8	73	A	G	G	99.9	SNP	3824	4375	8193	3823	4370	99.9	0.1	0	0	0	0.1	0	73G	73G	confirmed	99.9
9	263	A	G	G	99.6	SNP	697	563	1255	693	562	99.6	0.2	0.2	0	0	0.2	0	263G	263G	confirmed	99.6
10	290	A	_	_	94.1	DEL	669	607	1201	603	598	0	5.9	0	0	0	0	94.1	290del	290del	likely	94.1
11	291	A	_	_	97.9	DEL	669	607	1249	651	598	0	0.8	1.3	0.1	0	0	97.9	291del	291del	confirmed	97.9
12	309	T	+	C	23.9	INS	757	1113	446	87	359	0	0	0	0	0	25.3	0	309.1C	309.1C	likely	23.9
13	315	G	+	C	57.8	INS	747	1128	1083	69	1014	0	0	0	0	0	60.7	0	315.1C	315.1C	possible	57.8
14	489	T	C	C	99.5	SNP	1352	992	2333	1344	989	0	0	0.4	99.5	0	0.6	0	489C	489C	confirmed	99.5
15	16169	C	T	T	98.4	SNP	876	1382	2222	853	1369	0	0.1	98.4	1.1	0	0.3	0.4	16169T	16169T	confirmed	98.4
16	16223	C	T	T	99	SNP	1260	1926	3153	1243	1910	0	0	99	0.8	0	0.3	0.2	16223T	16223T	confirmed	99
17	16298	T	C	C	99.5	SNP	718	824	1535	717	818	0	0	0.5	99.5	0	3.4	0	16298C	16298C	confirmed	99.5
18	16325	T	C	C	99	SNP	706	824	1515	697	818	0	0.2	0.8	99	0	0.2	0	16325C	16325C	confirmed	99
19	16327	C	T	T	99.6	SNP	706	824	1524	705	819	0	0	99.6	0.4	0	0.5	0	16327T	16327T	confirmed	99.6
20	16642	A	G	G	99.9	SNP	2103	2220	4318	2100	2218	99.9	0.1	0	0	0	0.1	0	73G	73G	confirmed	99.9
21																						
22																						





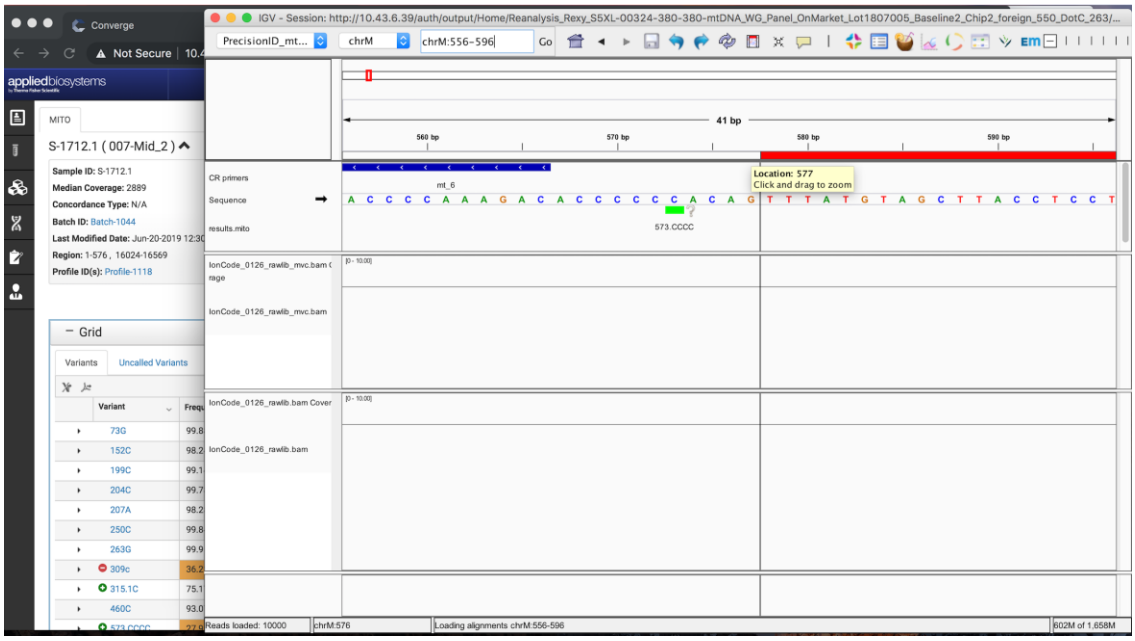
- Exported multi EMPOP file generated for Mito "Export Results" on the result card does have entries for all samples. This occurs when selected samples have more than 3 discrete regions as highlighted in the image below.



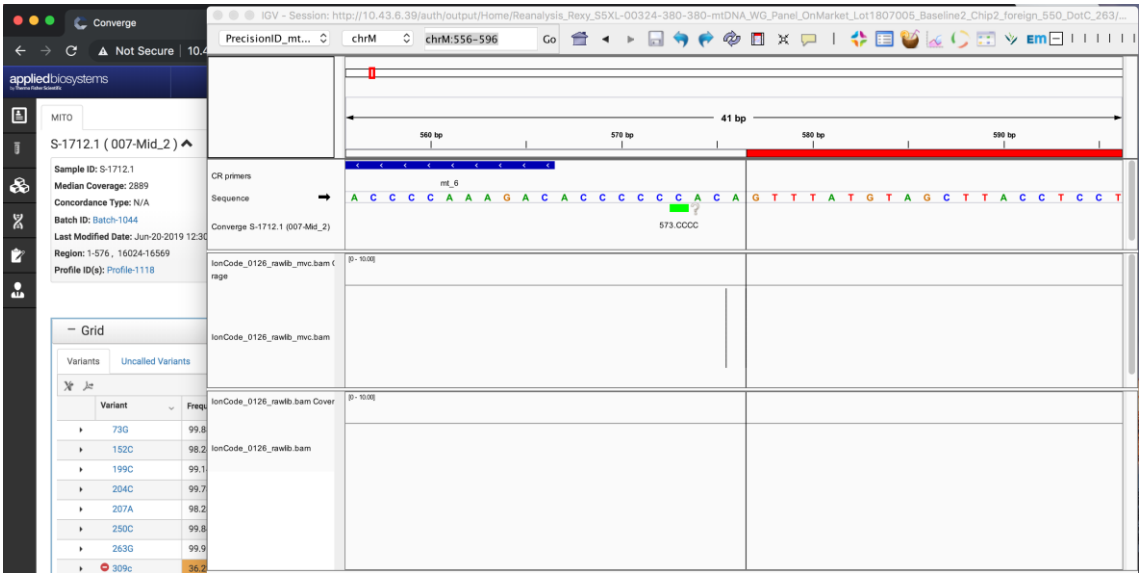
- In Mito IGV "Excluded regions" are incorrectly highlighted when variant is loaded on first instance. A second click on the variant is required to view the correct excluded region highlight in IGV.

  In the example below, the excluded region begins from position 577 but in IGV its highlighted from 578. A second click on the variant will highlight the excluded region from 577.

  First variant click instance:

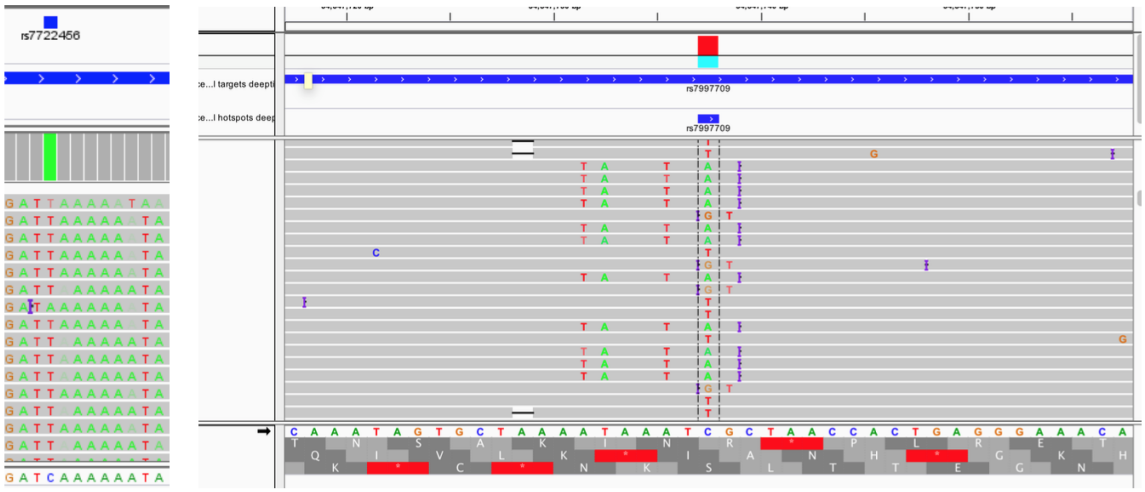


Second variant click instance:

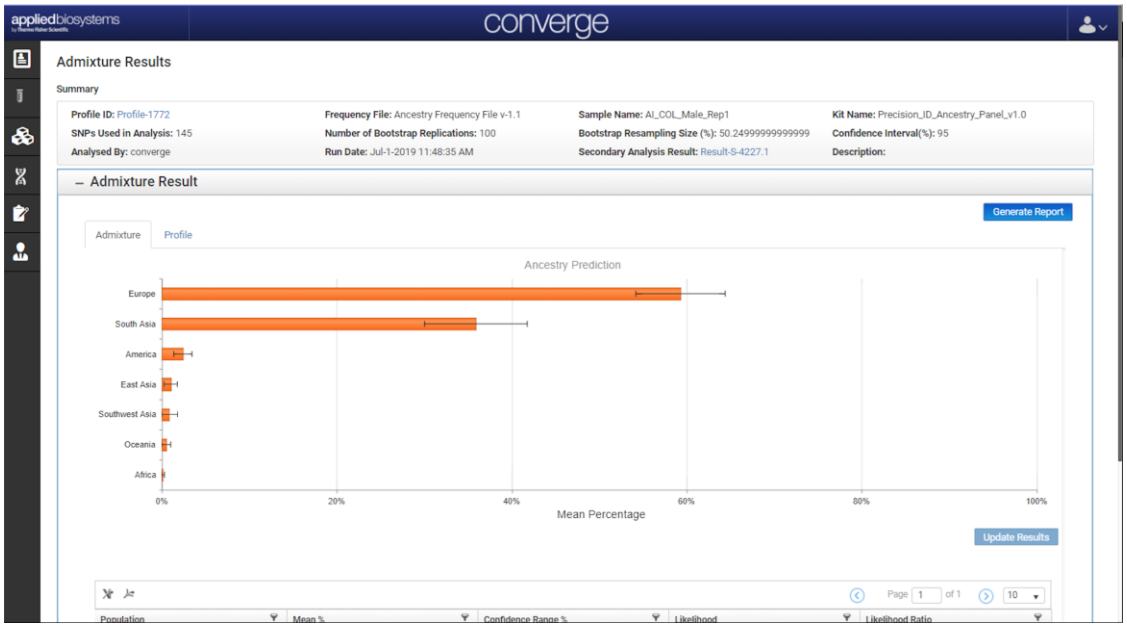


- Genotype errors may be observed in homopolymer regions with a continuous string of A bases. For example rs7722456 (aiSNP), which lies near a homopolymer region (6As; A bases incorrectly align with rs7722456 position & hence, "A" genotype overcall is added). Approximately 15% of reads show an "A" call as seen in the contig assembly snapshot in IGV Browser and could be due to a sequence artefact around homopolymer regions.

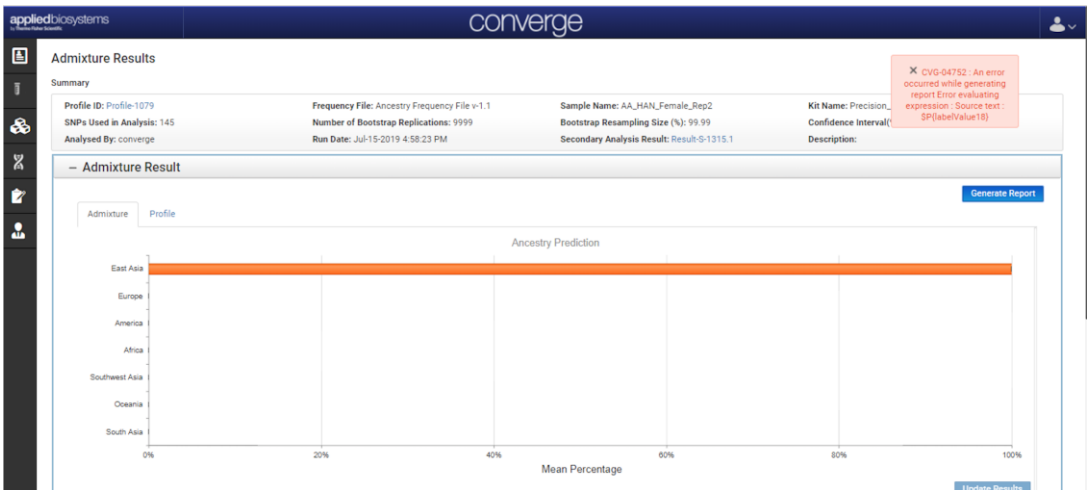
  Additional SNP id's with similar sequence artifacts follow:
  a. rs772262 – C is added to this SNP position because of poly Cs (5Cs)
  b. rs13400937 T is added to this SNP position because of poly Ts (4 Ts)
  c. rs7997709 A & G is added due to polyA region in the vicinity (snapshot below)

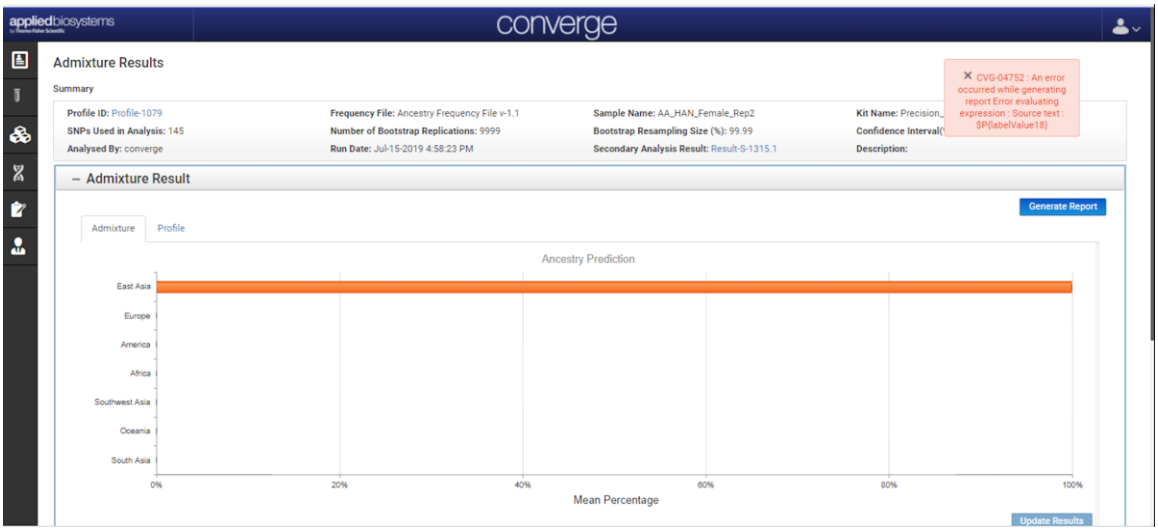


- For admixture analysis inputs, “Bootstrap Resampling Size (%)” parameter value 50.25 is automatically converted to 50.2499999999999 after analysis is complete.



- The limitation for report generation for admixture analysis is 9999 bootstrapping replications and 99.99 resampling size.





- In “Manage Kit” section while importing a kit with ~50 or more loci, there is a delay in response when the order of loci is changed.

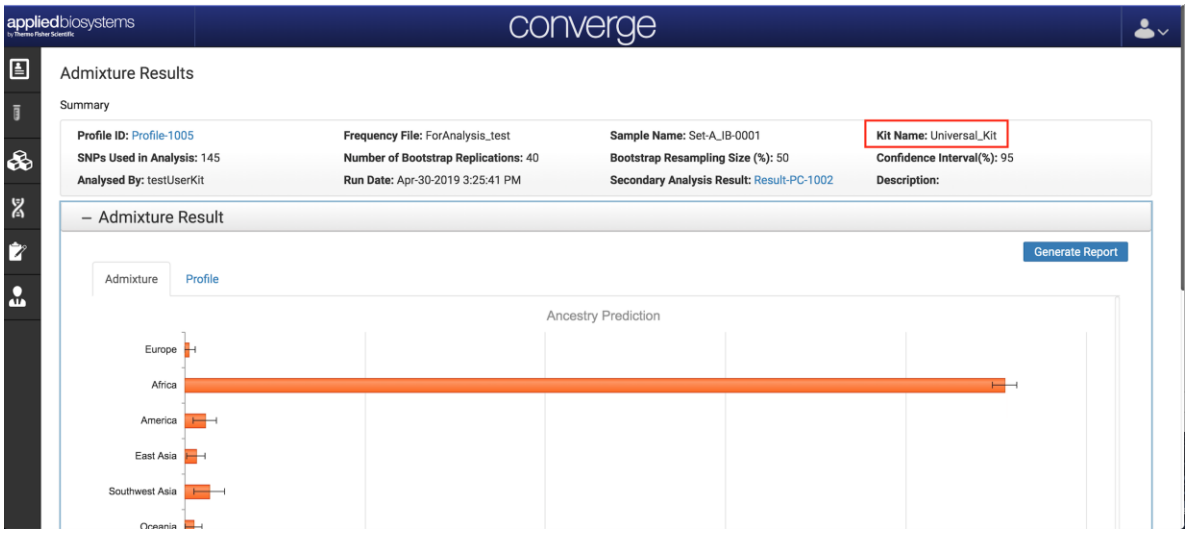
- Export of STR Results grid in pdf format shows SNPs in Flank (SIF) indicator (red dot) across markers that do not have SIFs.

Locus	Genotype	AN	OL	PHR	BST	ME	IGV
AMELX	1						
AMELY							
CSF1PO	6, 10, 11						
D10S1248	13, 14, 15						
D12ATA63	13, 14, 15, 15, 16						
D12S391	19, 20, 20, 20, 21, 21.1						
D13S317	9, 10, 12, 13						
D14S1434	13, 14						
D16S539	8, 9, 11, 12						
D18S51	16, 17, 18, 19						
D19S433	9, 10, 11						
D1S1656	13, 14, 16.3, 17.3						
D1S1677	14, 15						
D21S11	27, 28, 29, 30						
D22S1045	10, 11, 15, 16, 17						
D2S1338	17, 18, 18, 19						
D2S1776	11, 12						
D2S441	9, 10, 11						
D3S1358	14, 15						
D3S4529	14, 16						
D4S2408	10, 11						
D5S2800	13, 14, 19, 20						
D5S818	10, 11, 12						
D6S1043	10, 11, 12						
D6S474	13, 14, 15						
D7S820	7, 8, 9						
D8S1179	13, 14						
DYS391							

- Altered version of STR target.bed file is downloaded from HID Genotyper Result page.

  Target file download button downloads the "Processed" version of the STR target.bed file. This version has all the motif and insert coordinate information stripped from the file and cannot be re-uploaded and used by the user. The application does not deliver the "original" uploaded target.bed file.

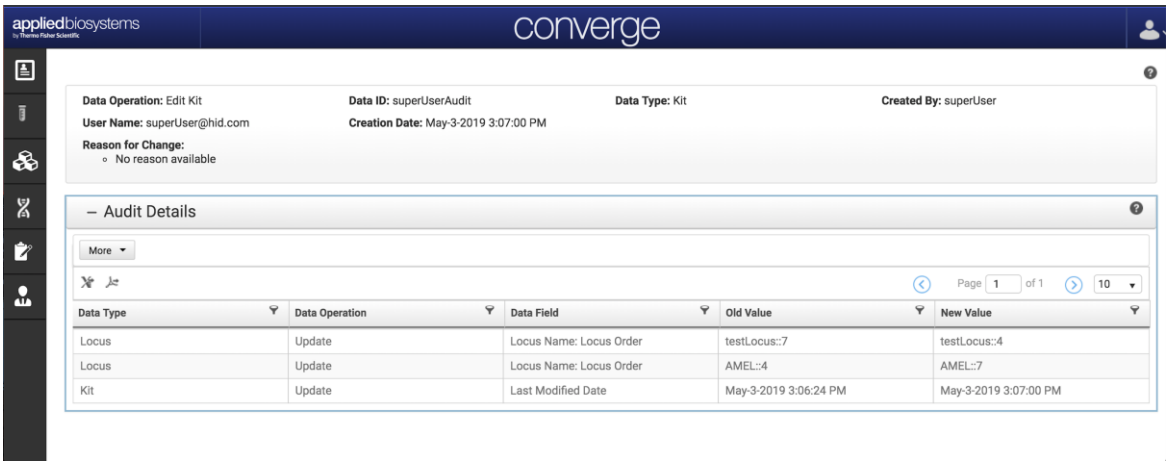
- On the Admixture summary page “Universal Kit” is displayed though a different kit is associated with Ancestry template.



- A warning message is displayed on launching IGV for SNP loci. IGV will be launched when user clicks on “OK”

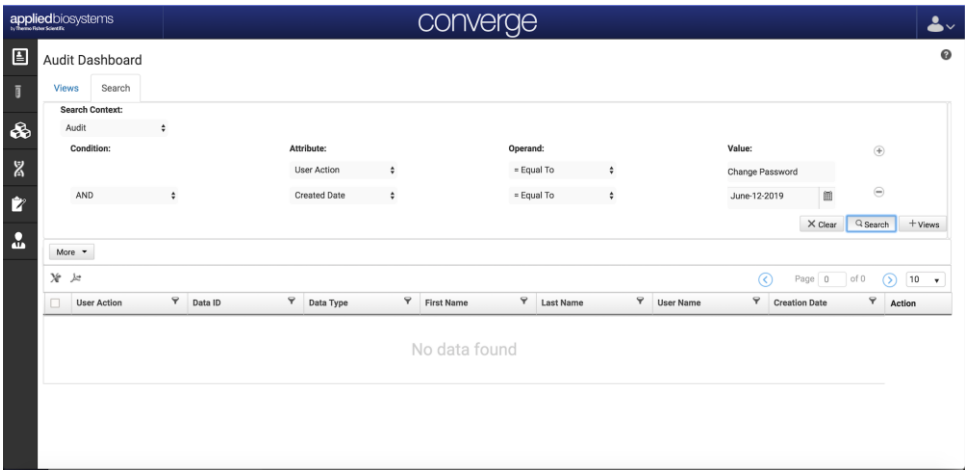
- The locus order in audit is incorrect when locus in the kit is deleted.

  For example: If there are 7 loci while uploading the kit, all except 4th and 7th loci are deleted then the remaining 2 loci should have 1 and 2 order respectively. Presently the initial loci order is created during uploading the kit is retained and not reindexed.

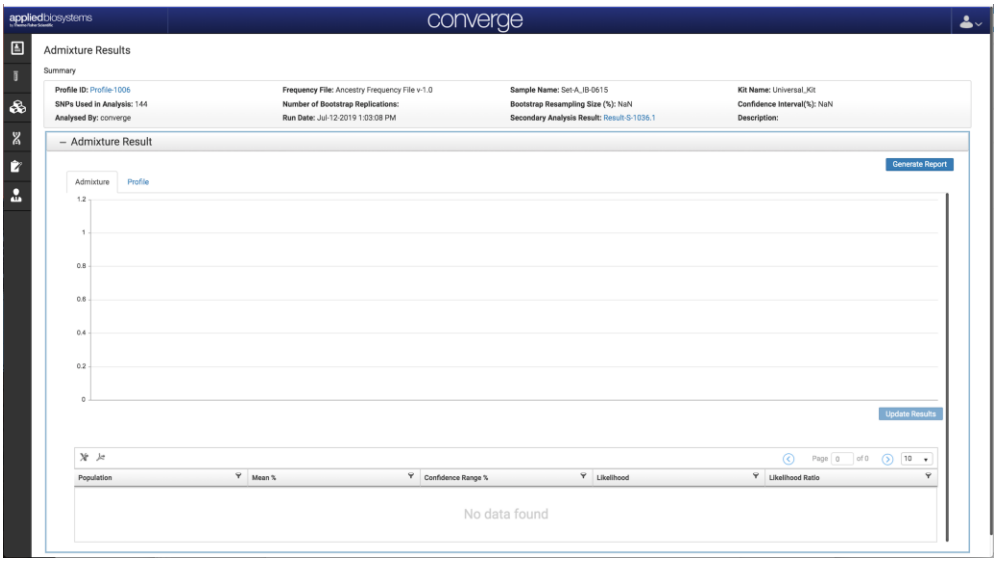


➢ **Upgrade related:**

- Audit Search with creation date "Equal to" operand in Converge 2.2 does not return any result.



- Admixture analysis results generated with Converge 2.1 version cannot be viewed on Converge 2.2 and results will be empty, as Converge 2.2 has Bootstrap method and related UI elements.



- Ancestry and Identity templates of Converge 2.1 will not be visible as the older versions (e.g. Converge 2.1, Ancestry 1.0 templates) are uninstalled during the upgrade process.

SOFTWARE RELEASE NOTES

applied biosystems by Thermo Fisher Scientific

RELEASE & COMPATIBILITY SUMMARY:

SYSTEM	TYPE	DESCRIPTION	VERSION / DATE STAMP
TSS	Software	TSS Compatibility	v5.10
	Publisher STR Control Files	Control_Samples	v1.01
		Precision_ID_GlobalFiler_NGS_STR_Control_Sample_male007	v1.1
		Precision_ID_GlobalFiler_NGS_STR_Control_Sample_9947A	v1.1
		Precision_ID_GlobalFiler_NGS_STR_Control_Sample_NegCtrl	v1.1
		Precision_ID_GlobalFiler_NGS_STR_Control_Sample_9947A_and_male007_and_NegCtrl	v1.1
TSS Control Publisher	NGS mtDNA Module	Precision_ID_mtDNA_Whole_Genome_Panel_Targets_v1.0.bed	v1.0
	STR Control Files	Precision_ID_mtDNA_Control_Region_Panel_Targets_v1.0.bed	v1.0
		Precision_ID_mtDNA_Panel_AnalysisParams_v1.0.json	v1.0
	NGS SNP Module	Precision_ID_Ancestry_Panel_Targets_v1.0.bed	v1.0
		Precision_ID_Ancestry_Panel_Hotspot_v1.0.bed	v1.0
BED/ JSON File	NGS mtDNA Module	Precision_ID_Identity_Panel_Target_v1.0.bed	v1.0
		Precision_ID_Identity_Panel_Hotspot_v1.0.bed	v1.0
	NGS STR Module	Precision_ID_GlobalFiler_NGS_STR_Panel_Target	v1.1
		Precision_ID_GlobalFiler_NGS_STR_Panel_Hotspot	v1.1
	NGS SNP Module	Precision_ID_GlobalFiler_NGS_STR_Panel_AnalysisParams	v1.1
HID Genotyper	Plugin Software	HIDGenotyper-2.2 (Serial No.)	2.2_4b0cd383b
		About (Serial No.)	4b0cd383b-201908300532
	Module	Platform	v2.2
	License	Case Management	v2.2
	Module	Kinship and Paternity Analysis	v1.0
	License	Converge Kinship	v1.0
	Module	NGS Application	v1.2
	License	Converge NGS	v1.2
Converge Software	Upgrade Installer Components	ConvergeUpgrade-2.2-0.x86_64.rpm (Serial No.)	4b0cd383b-201908300532
	Module	upgradecvg (Serial No.)	v1.0
	Supplemental Files	Converge-NGS-1.2.zip	v1.2
	Module	Precision_ID_Panel_Definitions.zip	Oct 15th 2018
	License	Precision_ID_mtDNA_rCRS File (NCBI reference NC_012920)	July 19th 2017