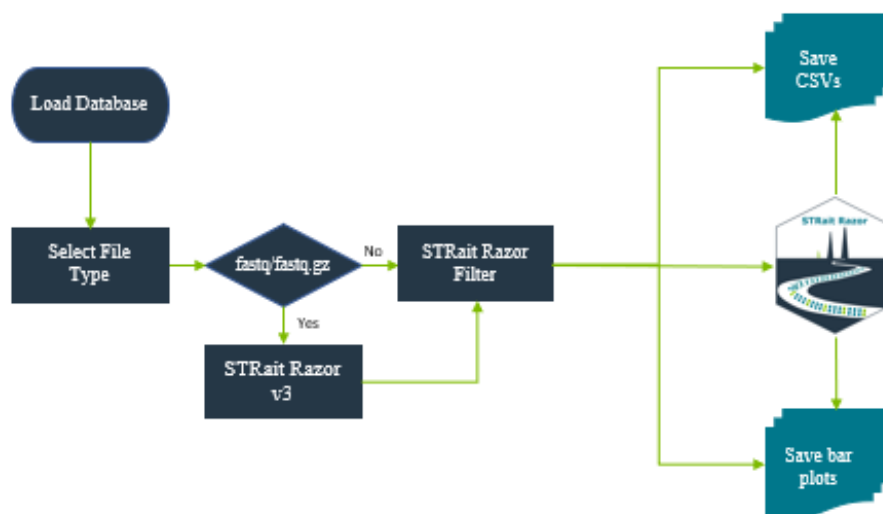


STRait Razor Online Manual



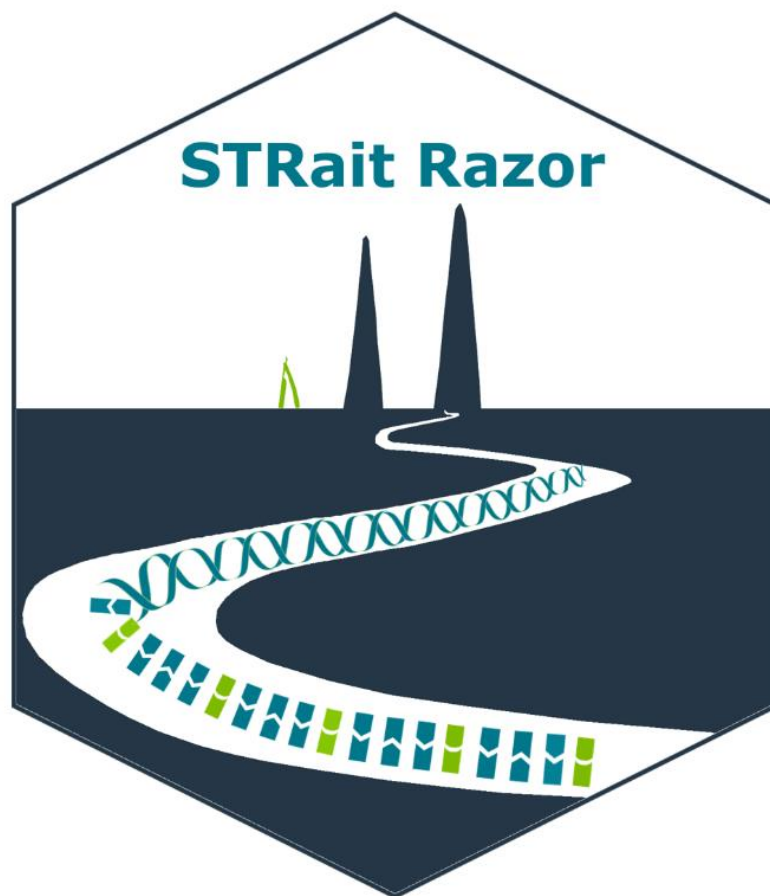
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Purpose

STRait Razor Online (SRO) serves as the user-interface (UI) for analyzing sequencing data with STRait Razor. Using the SRO, converts STRait Razor sequence-based allele calls into genotype tables for and/or bar plots for downstream analysis. STRait Razor Online also allows the user to control the genotype calls using several thresholds (e.g., heterozygous balance, strand balance, etc.). Read strands are merged and reported as single strand (Default = Forward) in Genotype Tables.*

*STRait Razor use is detailed in separate manual. Use of this workbook assumes a degree of STRait Razor knowledge prior to use.



Installation (Beta Test)

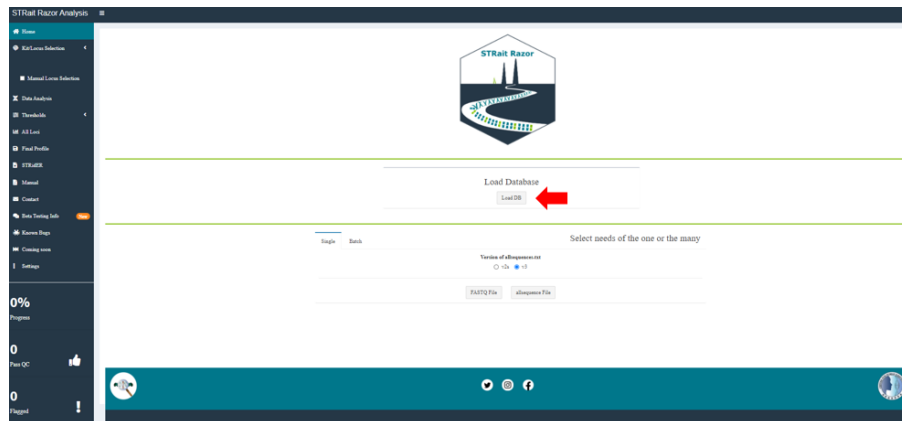
Congrats on joining us on this beta journey. It will be painful [in comparison to typing `install.packages("STRaitRazor")`]. But I sincerely thank you for your bravery. The final version will be much easier to bring online.

Note to **macOS** users: Please install Xquartz from <https://www.xquartz.org/> and then logout and log back in.

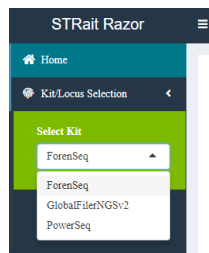
- 1) Download the package at the attached link.
- 2) Unzip the STRaitRazorOnline.zip file.
 - a. **Linux** users: rename str8rzt linux to str8rzt
 - b. **macOS** users: rename str8rzt osX to str8rzt
- 3) If you don't have R installed, install R from your mirror of choice <https://www.r-project.org/>
- 4) If you don't have RStudio installed, install RStudio Desktop from <https://rstudio.com/products/rstudio/download/#download>
- 5) Once you have R and RStudio installed, a few packages need to be installed. You can install these packages individually using the included STRait_Razor_Online_Installation.Rmd found in the ~\\STRAitRazorOnline\\ directory.
 - a. **Note:** These packages are Imports not Suggests. So, make sure you get them all. ☺
- 6) Once all the packages are installed, open 'app_standalone.R' script from the same directory as the SROInstallation.Rmd.

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Landing Page



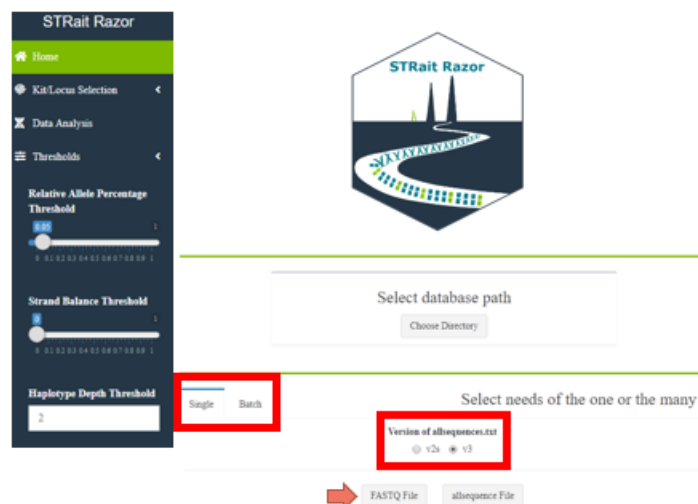
1. Load the database files into the environment using the 'Load DB' Button.
2. In sidebar, use the dropdown under 'Select Kit' based on the amplification primers* used.



***Note:** ForenSeq PMB is the default state for analysis.

3. Select 'Single' or 'Batch' Tab for analysis of one "sample" or ≥ 2 "samples".

Note: the process of compiling the list of fastq files can occur recursively or not (default = TRUE). If you have subdirectories with the same sample name, results may be overwritten 😞. See 'Settings' tab on sidebar to change.

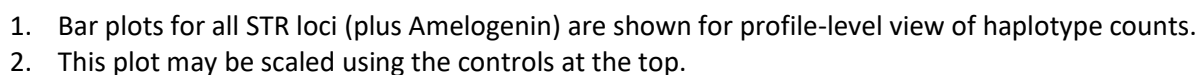


4. STRait Razor Online accepts both fastq/fastq.gz and allsequences.txt files from previous analyses to reduce analysis time of “reprocessing” fastq files. If processing allsequences.txt, please select ‘v2s’ or ‘v3’ format.

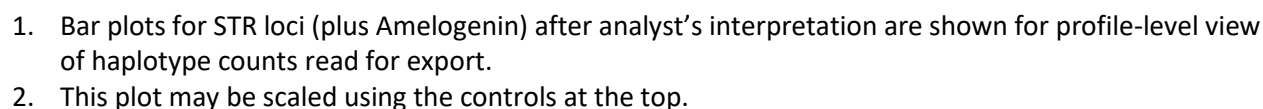
The screenshot displays the Strail Razor web application interface. The sidebar on the left includes navigation options: Home, Kat Locus Selection, Data Analysis (highlighted), Thresholds, Strand Balance Threshold, Haplotype Depth Threshold, All Loci, Final Profile, Manual, and Beta Testing Info. The main content area features two bar charts. The left chart, titled 'D10S1248', shows 'Read Depth' for alleles 10 and 11. The right chart, titled 'Called Alleles', shows 'Read Depth' for alleles 10 and 11. Below the charts are buttons for 'View Full Profile', 'Previous Locus', 'Test Locus', 'Next Locus', and 'Save Locus'. A table below the buttons shows the 'Final Profile' with columns for Status, SampleName, Locus, Allele, Name, Haplotype, HB, RAP, SB, and Haplotype. The table contains 4 rows of data for different samples and alleles. At the bottom, there are buttons for 'Clear Called Alleles Table' and 'Save Final Data Frame'.

1. Raw plot of reads above threshold
2. Thresholds
 - a. per locus
 - b. editable via input in sidebar (dynamically for UI only) or STRaitRazorAnalysis.config (static within an analysis; haplotypes passing conditionals create data frame for analysis; Appendix A)
3. Table of alleles > threshold
 - a. “Checking” the ‘Status’ of an allele will pass the result to the “Called Alleles” plot
4. Called Alleles bar plot
 - a. Final set of alleles
5. Autosave Data
 - a. “Checking” this will push called alleles to final data frame when you press ‘Next Locus’
6. Status Bar
 - a. In current version, status bar reflects loci with high Relative Allele Proportion (RAP) (e.g., Orange < AutoRAP). Release version will include more optimized elements. (See settings page for more info)
7. Other buttons
 - a. Most other buttons are self-explanatory

All Loci Bar Plot



Final Profile Bar Plot



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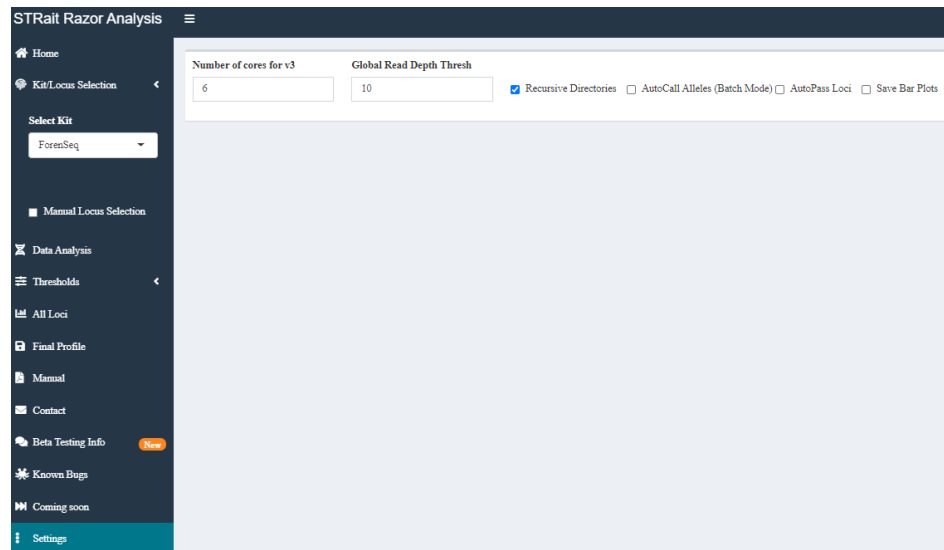
Manual

Have you ever seen the movie Inception? It is not quite the same. But it does launch a pdf of this doc.

Contact

Bat signal, but for data analysis help.

Settings




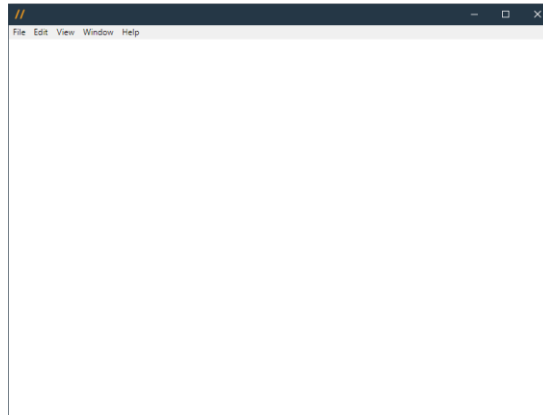
While this page will look significantly different with the final batch of settings added, this implementation controls a few different functions.


- Number of cores for v3**: Allocate system resources for fastq processing using str8zr program
- Global Read Depth Thresh**: Filter out haplotypes with fewer than X reads
- Recursive Directories**: When processing a batch of files (either fastq or allsequences), process chosen directory or directory plus subdirectories
- AutoPass Loci**: When processing single files, “passing” green loci are automatically moved to final data frame and “warning” orange loci are passed to ‘Data Analysis’ tab for interpretation (with some more optimization, will likely move this from Default = False → True)
- Save Bar Plots**: To save a .png of all loci bar plot to sample folder additionally a conditional setting for separating STR and SNP loci into separate image files

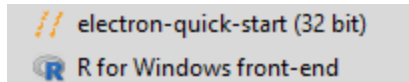
Troubleshooting

Windows Electron Application

- **Issue:** When launching electron app , users are met with blank screen.



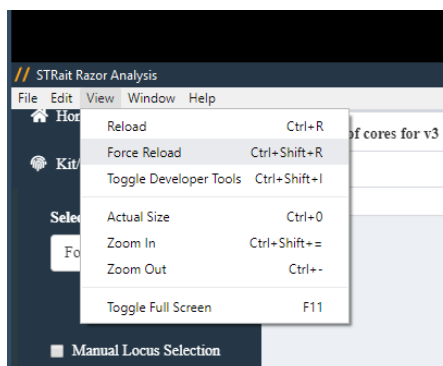
- **Solution:** View → Force Reload
- **Issue:** When launching electron app , users are met with error message “An error has occurred”.
- **Solution:** Open Task Manager and close any instances of electron-quick-start or R for Windows front-end. After these processes have been closed, relaunch the application.



- **Issue:** When selecting Settings → AutoPass Loci, Pass Loci Count changes to zero.



- **Solution:** View → Force Reload



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Appendix A: DatabasePath.csv

App	Path
STRaitRazorProgram	str8rzr
Analysis	STRaitRazorAnalysisConfig.csv
Haplotypes	HaplotypeDatabase.csv
ResultsParent	AnalysisOutput/
ResultsAnalysisChild	STRaitRazoR_AnalysisResults
DiploTypes	DiploTypeDatabase.csv
ResultsStutterChild	STRaitRazoR_StutterResults
Results_str8rzrChild	STRaitRazoR_FASTQ2TXT_Results
str8rzrConfig	STRaitRazorConfig.csv
DatabasePath	DatabasePath.csv

1. Database files and directory for fastq processing and analysis functions

Appendix B: STRaitRazorConfig.csv

Kitid	Path
ForenSeq	ForenSeqv1.24.config
GlobalFilerNGSv2	GFNGSv2_v6.config
mtDNA	mitoCstretcherv1.config
PowerSeq	PowerSeqv2.config

1. Configuration files for str8rzr application to analyze fastq/fast.gz files

Appendix C: HaplotypeDatabase.csv

Kitid	Locus	Haplotype	RepeatRegion	Flank	Motif	Nomenclature
ForenSeq	D5S818	TATTTATAC	ATCTATCTATCT		[ATCT]7	-GRCh38-chr5:123775543-123775606 [ATCT]7
ForenSeq	D5S818	TATTTATAC	ATCTATCTATCT		[ATCT]8	-GRCh38-chr5:123775543-123775606 [ATCT]8
ForenSeq	D5S818	TATTTATAC	ATCTATCTATCT	123775552-A	[ATCT]8	-GRCh38-chr5:123775543-123775606 [ATCT]8 123775552-A
ForenSeq	D5S818	TATTTATAC	ATCTATCTATCT		[ATCT]9	-GRCh38-chr5:123775543-123775606 [ATCT]9
ForenSeq	D5S818	TATTTATAC	ATCTATCTATCT	123775552-A	[ATCT]9	-GRCh38-chr5:123775543-123775606 [ATCT]9 123775552-A
ForenSeq	D5S818	TATTTATAC	ATCTATCTATCT		[ATCT]10	-GRCh38-chr5:123775543-123775606 [ATCT]10
ForenSeq	D5S818	TATTTATAC	ATCTATCTATCT	123775552-A	[ATCT]10	-GRCh38-chr5:123775543-123775606 [ATCT]10 123775552-A
ForenSeq	D5S818	TATTTATAC	ATCTATCTATCT		[ATCT]11	-GRCh38-chr5:123775543-123775606 [ATCT]11
ForenSeq	D5S818	TATTTATAC	ATCTATCTATCT	123775552-A	[ATCT]11	-GRCh38-chr5:123775543-123775606 [ATCT]11 123775552-A
ForenSeq	D5S818	TATTTATAC	ATCTATCTATCT		[ATCT]12	-GRCh38-chr5:123775543-123775606 [ATCT]12
ForenSeq	D5S818	TATTTATAC	ATCTATCTATCT	123775552-A	[ATCT]12	-GRCh38-chr5:123775543-123775606 [ATCT]12 123775552-A
ForenSeq	D5S818	TATTTATAC	ATCTATCTATCT		[ATCT]13	-GRCh38-chr5:123775543-123775606 [ATCT]13
ForenSeq	D5S818	TATTTATAC	ATCTATCTATCT	123775552-A	[ATCT]13	-GRCh38-chr5:123775543-123775606 [ATCT]13 123775552-A
ForenSeq	D5S818	TATTTATAC	ATCTATCTATCT		[ATCT]3 ATGT [ATCT]9	-GRCh38-chr5:123775543-123775606 [ATCT]3 ATGT [ATCT]9
ForenSeq	D5S818	TATTTATAC	ATCTATCTATCT		[ATCT]14	-GRCh38-chr5:123775543-123775606 [ATCT]14
ForenSeq	D5S818	TATTTATAC	ATCTATCTATCT	123775552-A	[ATCT]14	-GRCh38-chr5:123775543-123775606 [ATCT]14 123775552-A
ForenSeq	D5S818	TATTTATAC	ATCTATCTATCT		[ATCT]3 ATGT [ATCT]10	-GRCh38-chr5:123775543-123775606 [ATCT]3 ATGT [ATCT]10
ForenSeq	D5S818	TATTTATAC	ATCTATCTATCT		[ATCT]15	-GRCh38-chr5:123775543-123775606 [ATCT]15

This database file contains haplotypes with associated metadata (i.e., flanking region SNPs, repeat region motif, and region bounds).

Note: The SNPs are largely annotated by position. Ultimately, these will be migrating to rs#.

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Appendix D: STRaitRazorAnalysisConfig.csv

KitId	Order	Locus	RefSeq	Repeat_Size	Motif_Spacer	HG38_Allele	chr	FRStart	FRStop	RRStart	RRStop	RRStart	RRStop	Repeat_Region_Start	Repeat_Region_Stop	HBT	RDT	SBT	RAPT	AutoRAPT	MV_AlignAdjust	InternalMotif1	InternalMotif2	InternalMotif3	Marker_Type
PowerSeq	0	Amelogenin		6												0.4	2	0	0.001	0.6		TCAAGT	-	-	Indel
PowerSeq	8	D1S1656	GAAATA	4	TCTACATC	17	chr1	230769561	230769704	230769616	230769683			230769616	230769683	0.4	2	0.01	0.001	0.6	TCA:TC A;	TCTATCTATCTA	-	-	STR
PowerSeq	11	D2S1338	CTAGCA	4	GGCAAGGC	23	chr2	218014794	218014998	218014853	218014950			218014853	218014950	0.4	2	0	0.001	0.6	TGAT:T GAT;	GGAAGGAAGGAA	-	-	STR
PowerSeq	12	D2S441	TGCACC	4	TCTATATC	12	chr2	68011866	68011939	68011947	68011934			68011947	68011934	0.4	2	0.01	0.001	0.6	TCA:TC A;	TCTATCTATCTA	-	-	STR
PowerSeq	21	TPOX	CACTGG	4	AATGTTTG	8	chr2	1489529	1489638	1489653	1489684			1489653	1489684	0.4	2	0.01	0.001	0.6		AATGAATG	-	-	STR
PowerSeq	13	D3S1358	TGCCCA	4	TCTATGAG	16	chr3	45540639	45540824	45540739	45540802			45540739	45540802	0.4	2	0.01	0.001	0.6		ATCTATCTATCT	-	-	STR
PowerSeq	17	FGA	GTCTGA	4	XXXXXXXX	22	chr4	154587656	154587823	154587736	154587823			154587736	154587823	0.4	2	0	0.001	0.6	AAAGAAAAAGA	AAAGAAAG	-	-	STR
PowerSeq	1	CSF1PO	CTAAGT	4	ATCTAATC	13	chr5	150076311	150076487	150076324	150076375			150076324	150076375	0.4	2	0.01	0.001	0.6		ATCTATCTATCT	-	-	STR
PowerSeq	14	D5S818	AACATT	4	ATCTTCAA	11	chr5	123775517	123775680	123775556	123775599			123775556	123775599	0.4	2	0.01	0.001	0.6		ATCTATCTATCT	-	-	STR
PowerSeq	15	D7S820	AGAATT	4	TATCGTTA	13	chr7	84160149	84160346	84160226	84160277			84160226	84160277	0.4	2	0.01	0.001	0.6		TATCTATC	-	-	STR
PowerSeq	16	D8S1179	TTTCATC	4	TCTATTCC	13	chr8	124894846	124895024	124894865	124894916			124894865	124894916	0.4	2	0.01	0.001	0.6		TCTATCTATCTA	-	-	STR
PowerSeq	2	D10S1248	CCCCAG	4	XXXXXXXX	13	chr10	129294190	129294295	129294244	129294295			129294244	129294295	0.4	2	0	0.001	0.6		GGAAGGAAGGAA	-	-	STR
PowerSeq	20	TH01	CTCCATC	4	AATGAGGG	7	chr11	2171078	2171277	2171088	2171115			2171088	2171115	0.4	2	0.01	0.001	0.6	TGAT:TG AT;	ATGAATGA	-	-	STR

For this implementation, we will be focusing on the columns currently implemented [graphite shaded].

Kit: Amplification kit(s) used for target enrichment*

***Note:** ForenSeq PMB is the default state for analysis.

Locus: Full list of markers in each kit

Repeat Size: Period of the repeat (e.g., CSF1PO: ATCT; 4 base repeat)

HBT: Heterozygote Balance Threshold on a per marker & per kit basis. This is used for assignment of second allele prior to data frame passing to UI

Danger_math_ahead: The threshold is calculated by dividing the second largest, in terms of coverage or read depth, by the largest allele (e.g., the allele 12 has 932 reads associated with it and allele 17.3 has 950 reads. Thus, the heterozygote balance is 0.98). However, the heterozygote balance output in the genotype tables is calculated by dividing the largest (lexicographically) allele by the second largest allele (e.g., $\frac{G}{A}$, or $\frac{14}{10}$).

RDT: Read Depth Threshold on a per marker & per kit basis filter prior to data frame passing to UI

SBT: Strand Balance Threshold on a per marker & per kit basis filter prior to data frame passing to UI

RAPT: Relative Allele Proportion Threshold on a per marker & per kit basis filter prior to data frame passing to UI

AutoRAPT: Locus flagging variable. In this beta, loci with a proportion of called alleles above this value will be flagged “green” (e.g., CSF1PO; 1000 reads aligned to the locus, 12: 450, 14: 450, AutoRAPT = 0.857; 900/1000 == 0.90; 0.9 > 0.857, CSF1PO = “green”)

Marker Type: Categories of marker type (e.g., SNP, microhaplotype, STR) used for parsing markers

Appendix E: DiplotypeDatabase.csv

Under Construction

Check Back Soon

Appendix F: RepeatRegion.csv

Under Construction

Check Back Soon

Appendix G: RepeatRegion_MM.csv

Under Construction

Check Back Soon

Changelog

v 0.1.2: **07/20/2020**

- Launch Day!!!!!!!!!!!!

v 0.1.3: **08/19/2020**

- minor bug fixes

v 0.1.4: **09/01/2020**

- added STRidER tab
- STRidER input fields
- added CV to RAP batch output
- fixed bug affecting SNP loci with secondary SNP variant in matching sequence
- added progress bar for batch mode or file processing

v 0.1.5: **09/17/2020**

- added data.table package to address STRidER appending issue
- finalized STRidER functions for multi-sample processing
- cleaned up in script comments regarding page titles
- removed readxl package (not used in current implementation)
- added code to clean up UI on start-up
- Launched Windows Electron application
- added function subThresh for saving data frame of reads \geq GlobalThreshold, but $<$ locus threshold
- added Indels (e.g., Amelogenin) to All Loci and Final Profile ggplots

v 0.1.51: **09/18/2020**

- corrected bug for fastq processing related to relative path of configs in unzipped fastq pipeline (app_online.R only)

v 0.1.6: **11/10/2020**

- adjusted calculation of heterozygote balance for isoalleles and SNP loci
- added toggle switch for expected vs. observed
- adjusted calculation of progress box
- added spacer box for missing loci when cycling through under data analysis
- modified allele reporting to SNP/Amel to character rather than numeric in table output
- removed closed beta tabs

v 0.1.7 (config update...no code): 01/05/2021

- Updated recommended PowerSeq config v2-->v2.1