

Kongoh version 3.3.0 User Manual

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Information on Kongoh

Kongoh (named after the Japanese word “mixture”) is open-source software for DNA evidence interpretation based on a quantitative continuous model[1, 2]. The software is a graphical user interface and has been released as the R-package *Kongoh* (>= ver. 3.2.0).

Kongoh can perform mixture deconvolution and the assignment of the likelihood ratio for crime stain profiles typed by the AmpFlSTR® Identifiler® Plus PCR Amplification Kit (Thermo Fisher Scientific, Waltham, MA) and GlobalFiler™ PCR Amplification Kit (Thermo Fisher Scientific). Profiles typed by kits other than Identifiler Plus and GlobalFiler can be analyzed if experimental data are prepared from the kit. A peak located at the position of the back stutter, forward stutter, double-back stutter, and minus 2-nt stutter need not be designated as an allele or stutter because the derivation of the peak in the stutter position can be determined probabilistically. Hence, the stutter filters of all loci can be removed. Moreover, Kongoh considers allelic drop-out, which is the event of a peak under the analytical threshold. By contrast, drop-in is not considered; however, spontaneous drop-in peaks can be explained based on additional unknown contributors.

Version changes

Note

Users are recommended to switch to version 3.3.0 because of the following updates.

Changes in ver. 3.3.0

New features

- The likelihood ratio can be calculated based on the result of mixture deconvolution.

Important update

- An issue caused by a function for excluding unrealistic genotype combinations was fixed. In some cases, the mixture ratio filter did not work correctly.

Changes in ver. 3.2.3

Important update

- An important bug was fixed. The displayed information on genotype combinations and the weight values in the "Likelihood ratio" tab was not correct when at least one allele in reference profiles was dropped out. This is a display issue only. The update does not affect the result of the mixture deconvolution and the likelihood ratio.

Changes in ver. 3.2.2

Important updates

- A calculation error was fixed. In the previous version of Kongoh, the error occurred in the case that one of the expected forward stutter ratios (FSRs) or the expected double-back stutter ratios (DSRs) were not greater than zero under the model for these stutters. In this case, the new version of Kongoh does not regard the observed peak at the stutter position as only the stutter product.
- A calculation error was fixed. In the previous version of Kongoh, the error occurred when there were some locus names with spaces (e.g., Penta D and Penta E) in the input files.
- An error about setting analytical thresholds in the "Set analysis methods" was fixed.

Minor changes

- The default file "GF_29cycles_3500xL_24sec.csv" is updated. The update does not affect the result of the mixture deconvolution and the likelihood ratio.

Changes in ver. 3.2.1

New features

- The likelihood ratio is displayed on both normal and logarithmic scales in the "Result" of the "Likelihood ratio" tab.
- Users can export likelihoods and LRs of all assumed hypotheses.

Minor changes

- The term "Likelihood" has been changed to "Log10 (Likelihood)" in the "Result" of the "Deconvolution" tab.
- The following minor issue was fixed. The unexpected error message "Error: The total number of contributors is smaller than the number of known contributors." was displayed when the assumed number of contributors was smaller than the number of reference profiles inputted.

Changes in ver. 3.2.0

New features

- Kongoh can be used as the R-package *Kongoh*.
- UI design has been changed.
- Project data can be saved and loaded.
- A new feature "mixture deconvolution" has been implemented. In this feature, probabilistic genotyping can be performed without reference profiles.
- The expected peak height of drop-out alleles (Q) is regarded as that of the most frequent allele in Q regardless of known and unknown contributors.
- A new feature "Set analysis methods" has been implemented.
 - Users can set analysis methods per typing kit in the software.
 - Analytical thresholds can be set per locus.
 - The "mixture ratio filter" is added to exclude unrealistic genotype combinations.
 - The files for Monte Carlo parameters and the information on allele repeat correction can be specified when setting analysis methods. Therefore, users no longer need to load these files in the "Files" tab.
- The file format for Monte Carlo parameters is changed as follows.

- Names of parameters in each model are explicitly declared in the column.
- The scale of "Mean" parameters for AE and Hb is changed from a logarithmic scale to a linear scale.
- See example files for GlobalFiler (GF_29cycles_3500xL_24sec.csv) and Identifiler Plus (IDP_28cycles_3130xl_10sec.csv) which are located at extdata > parameters in the package *Kongoh*.
- A new feature "Edit repeat correction" has been implemented. Users can edit information on allele repeat correction used for models of stutter ratios.
- The default files of allele repeat correction are updated. See files for GlobalFiler (GF_repeat_correction.csv) and Identifiler Plus (IDP_repeat_correction.csv) which are located at extdata > repeat_correction in the package *Kongoh*.
- When the input allele frequencies are represented as allele counts, the input frequencies can be viewed in both the format of allele counts and the format of allele probabilities estimated by Dirichlet distribution.
- Estimated gamma distributions are replaced with the expected peak heights of gamma distributions in the graph of probabilistic genotyping.
- There are some improvements in the feature "Estimate parameters".
 - Project data can be saved and loaded.
 - The information on "Sample File" and "Dye" in the file of experimental data is no longer needed. See the example file "Experimental-data_GF_Example.csv" which is located at extdata > example in the package *Kongoh*.
 - Parameters of min AE, min Hb, max BSR, max FSR, max DSR, and max M2SR can be determined based on the experimental data.
 - Functions for checking input files have been updated to detect the difference in the locus set and the sample names between each file.
 - The graph for stutter ratios has been improved when the option "Multiple loci together" is selected for the method of modeling.
- A new feature "Set information on typing kits" has been implemented. Users can create or edit information on the locus set, alleles, and sizes of each allele.

Minor changes

- The term "mixture ratio" has been changed to "mixture proportion".

- The term "Weight" has been changed to "Weight (0-1 scale)" in the result of probabilistic genotyping.
- An error message has been improved when there is a lack of information about allele repeat correction for the representative of the unobserved alleles (Q).

Tutorial

Getting started

1. Ensure that R ($\geq 4.2.0$) is installed. It is available from the R Development Core Team website (<http://www.R-project.org>).
2. Begin an R session.
3. Execute the following command in R to install required packages.

```
install.packages(c("tcltk2", "gtools", "truncnorm", "GenSA"))
```

4. Go to <https://github.com/manabe0322/Kongoh/releases>.

5. Download "Kongoh_3.3.0.zip".

Kongoh v3.3.0 Draft



Note

Users are recommended to switch to version 3.3.0 because of the following update.

Changes in v3.3.0

New feature

- The likelihood ratio can be calculated based on the result of mixture deconvolution.

Important update

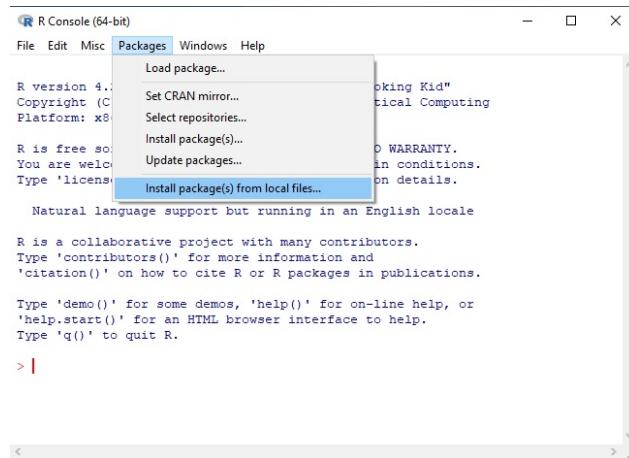
- An issue caused by a function for excluding unrealistic genotype combinations was fixed. In some cases, the mixture ratio filter did not work correctly.

Information on the previous version changes is shown in the user manual.

▼ Assets 3

| | | |
|----------------------|---------|--------------|
| Kongoh_3.3.0.zip | 6.57 MB | 1 minute ago |
| Source code (zip) | | 2 days ago |
| Source code (tar.gz) | | 2 days ago |

6. Install "Kongoh_3.3.0.zip" from "Install package(s) from local files...".

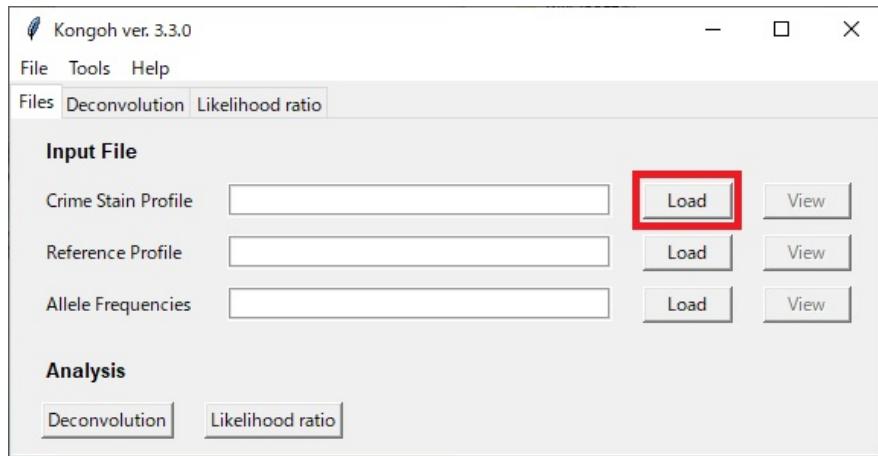


7. Execute the following commands in R to start GUI.

```
library(Kongoh)  
Kongoh()
```

Input files

1. Load a file of the crime stain profile.



Note

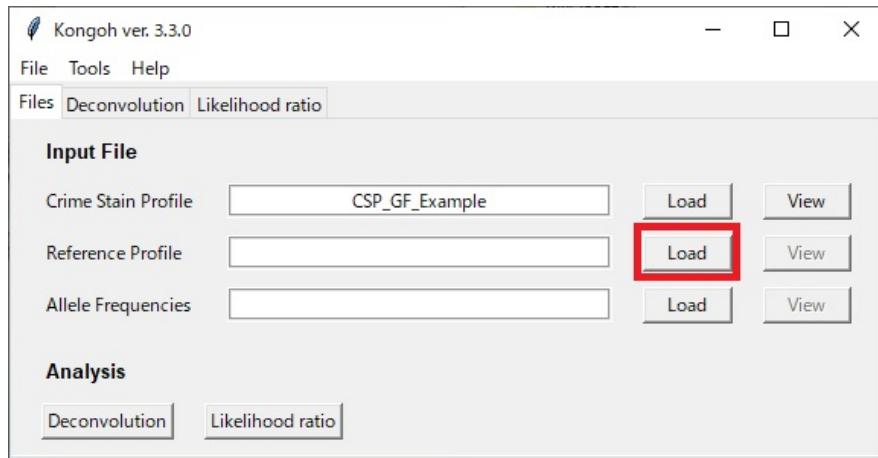
- The example file "CSP_GF_Example.csv" is provided. Go to extdata > example in the package *Kongoh*.
- This file can be exported from the GeneMapper® ID-X software.
- This file must include information regarding the "Sample Name", "Marker", "Allele", "Size", and "Height" as shown in Figure 1.
- Back stutters, forward stutters, double-back stutters, and minus 2-nt stutters are not necessarily removed manually¹.
- Pull-up peaks and noises must be removed manually.

¹Whether these stutters should be removed depends on the setting of the Monte Carlo parameters.

| Sample Name | Marker | Allele 1 | Allele 2 | Allele 3 | | Size 1 | Size 2 | Size 3 | | Height 1 | Height 2 | Height 3 | |
|-------------|----------|----------|----------|----------|--|--------|--------|--------|--|----------|----------|----------|--|
| Example | D3S1358 | 15 | 16 | 17 | | 121.35 | 125.35 | 129.39 | | 1800 | 262 | 1494 | |
| Example | vWA | 13 | 14 | 15 | | 164.95 | 169.06 | 172.92 | | 46 | 1640 | 93 | |
| Example | D16S539 | 8 | 9 | 10 | | 239.82 | 243.93 | 247.93 | | 137 | 1311 | 3200 | |
| Example | CSF1PO | 8 | 9 | 10 | | 291.15 | 295.15 | 299.07 | | 59 | 1831 | 165 | |
| Example | TPOX | 8 | 10 | 11 | | 350.77 | 359.01 | 362.95 | | 1150 | 2395 | 1100 | |
| Example | Yindel | | | | | | | | | | | | |
| Example | AMEL | X | | | | 98.81 | | | | 11136 | | | |
| Example | D8S1179 | 11 | 12 | 13 | | 138.5 | 142.62 | 146.78 | | 262 | 3557 | 4117 | |
| Example | D21S11 | 28 | 29 | 30 | | 199.64 | 203.65 | 207.57 | | 1715 | 79 | 2502 | |
| Example | D18S51 | 13 | 14 | 17 | | 285.76 | 289.76 | 301.73 | | 400 | 7163 | 1908 | |
| Example | DYS391 | | | | | | | | | | | | |
| Example | D2S441 | 9 | 10 | 11 | | 80.86 | 84.99 | 89.15 | | 70 | 1526 | 1500 | |
| Example | D19S433 | 12 | 12.2 | 13 | | 141.6 | 143.55 | 145.59 | | 134 | 61 | 2321 | |
| Example | TH01 | 6 | 7 | 8 | | 187.24 | 191.31 | 195.31 | | 922 | 929 | 628 | |
| Example | FGA | 18 | 19 | 21 | | 243.46 | 247.67 | 255.6 | | 63 | 1731 | 137 | |
| Example | D22S1045 | 15 | 16 | 17 | | 109.42 | 112.39 | 115.34 | | 1508 | 155 | 3497 | |
| Example | D5S818 | 8 | 9 | 10 | | 142.7 | 146.78 | 150.87 | | 55 | 1657 | 169 | |
| Example | D13S317 | 7 | 8 | 9 | | 206.87 | 210.82 | 214.81 | | 2071 | 1955 | 3971 | |
| Example | D7S820 | 8 | 9 | 10 | | 270.6 | 274.68 | 278.69 | | 58 | 1656 | 3747 | |
| Example | SE33 | 18 | 18.2 | 19 | | 362.31 | 364.3 | 366.36 | | 182 | 47 | 1940 | |
| Example | D10S1248 | 13 | 14 | 15 | | 106.04 | 110.06 | 114.08 | | 231 | 2959 | 2107 | |
| Example | D1S1656 | 14 | 14.2 | 15 | | 180.11 | 182.01 | 184.23 | | 190 | 46 | 4297 | |
| Example | D12S391 | 17 | 18 | 19 | | 228.52 | 232.48 | 236.46 | | 161 | 2919 | 184 | |
| Example | D2S1338 | 18 | 19 | 20 | | 309.14 | 313.13 | 317.35 | | 214 | 892 | 2961 | |

Figure 1: Format of the crime stain profile

- Load a file of the reference profiles.



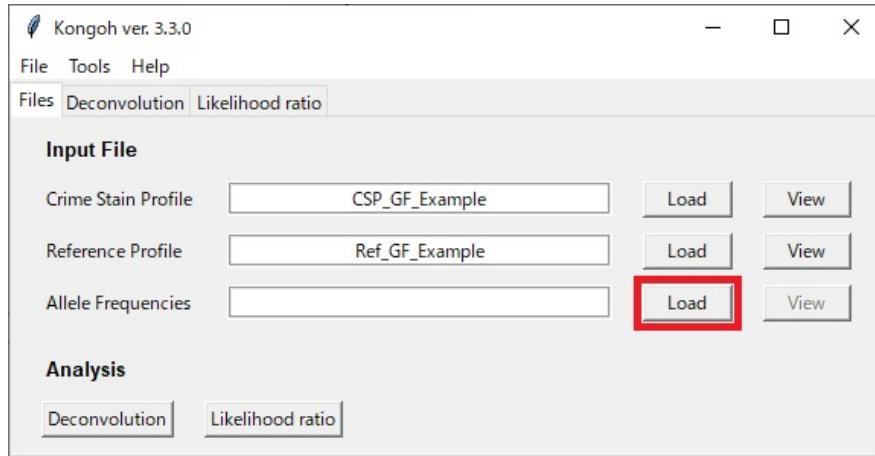
Note

- The example file "Ref_GF_Example.csv" is provided. Go to extdata > example in the package *Kongoh*.
- This file must include information regarding the "Marker" and the name of each profile (e.g., victim and suspect), as shown in Figure 2.
- Two alleles in homozygotes must be entered in each column.

| Marker | victim | victim | suspect | suspect |
|---------|--------|--------|---------|---------|
| D3S1358 | 15 | 18 | 17 | 19 |
| vWA | 16 | 17 | 14 | 18 |
| D16S539 | 10 | 10 | 9 | 13 |
| CSF1PO | 9 | 12 | 11 | 12 |
| TPOX | 10 | 10 | 8 | 11 |
| Yindel | | | | |
| AMEL | X | X | X | X |
| D8S1179 | 13 | 13 | 12 | 14 |
| D21S11 | 28 | 30 | 30 | 32.2 |
| D18S51 | 14 | 14 | 14 | 17 |
| DYS391 | | | | |
| D2S441 | 11 | 14 | 10 | 12 |

Figure 2: Format of the reference profiles

3. Load a file of the allele frequencies.



Note

- Users can input two formats of allele frequencies: the allele counts (Figure 3) and the allele probabilities (Figure 4).
- If the allele frequencies are represented as the allele counts, the probabilities of each allele are determined by the expected values based on the Dirichlet distribution.
- If the allele frequencies are represented as the probabilities, these values are directly used for the probabilities of each allele. When a crime stain profile or the reference profiles contain alleles that are not listed in the input file, then the minimum allele frequency is used as the frequency of these alleles.
- The allele frequencies for the Identifiler[3] and the GlobalFiler[4] in the Japanese population are provided. Go to extdata > example in the package *Kongoh*. The allele frequencies for the Identifiler are represented as the probabilities. The allele frequencies for the GlobalFiler are represented as the allele counts.

| Allele | D3S1358 | vWA | D16S539 | CSF1PO | TPOX | D8S1179 | D21S11 | D18S51 | D2S441 | D19S433 | TH01 | FGA | D22S1045 | D5S818 | D13S317 |
|--------|---------|------|---------|--------|------|---------|--------|--------|--------|---------|------|-----|----------|--------|---------|
| 5 | | | | | | | | | | | 4 | | | | |
| 6 | | | | | | | | | | | 663 | | | | |
| 7 | | 1 | 37 | | | 1 | | | | | 1 | 811 | | 8 | 6 |
| 8 | | 5 | 3 | 1357 | | | | | | 7 | | 194 | | 18 | 786 |
| 8.1 | | | | | | | | | | 1 | | | | | |
| 9 | | 1077 | 151 | 354 | 8 | | | | | | 1190 | | | 275 | 392 |
| 9.1 | | | | | | | | | | 110 | | | | | |
| 9.2 | | | | | | | | | | | 1 | | | | |
| 9.3 | | | | | | | | | | | 113 | | | | |
| 10 | | 602 | 648 | 100 | 387 | | | 6 | 805 | | | 27 | | 616 | 338 |
| 10.1 | | | | | | | | | | 7 | | | | | |
| 10.2 | | | | | | | | | | | 3 | | | | |
| 10.3 | | | | | | | | | | | | | | | |
| 11 | | 562 | 620 | 1072 | 319 | | | 14 | 1034 | 10 | | | 595 | 865 | 675 |
| 11.1 | | | | | | | | | | 1 | | | | | |
| 11.2 | | | | | | | | | | | 2 | | | | |
| 11.3 | | | | | | | | | | 68 | | | | | |
| 12 | 6 | 517 | 1267 | 114 | 370 | | | 140 | 511 | 132 | | | 4 | 692 | 608 |
| 12.2 | | | | | | | | | | | 13 | | | | |
| 13 | 3 | 1 | 209 | 208 | 3 | 678 | | 598 | 145 | 874 | | | 5 | 497 | 158 |
| 13.2 | | | | | | | | | | 89 | | | | | |
| 14 | 79 | 586 | 26 | 52 | 2 | 625 | | 643 | 298 | 1038 | | | 7 | 28 | 37 |
| 14.1 | | | | | | | | | | 250 | | | | | |
| 14.2 | | | | | | | | | | | | | | | |
| 15 | 1192 | 78 | 3 | 14 | | 410 | | 493 | 14 | 160 | | | 934 | 3 | 2 |

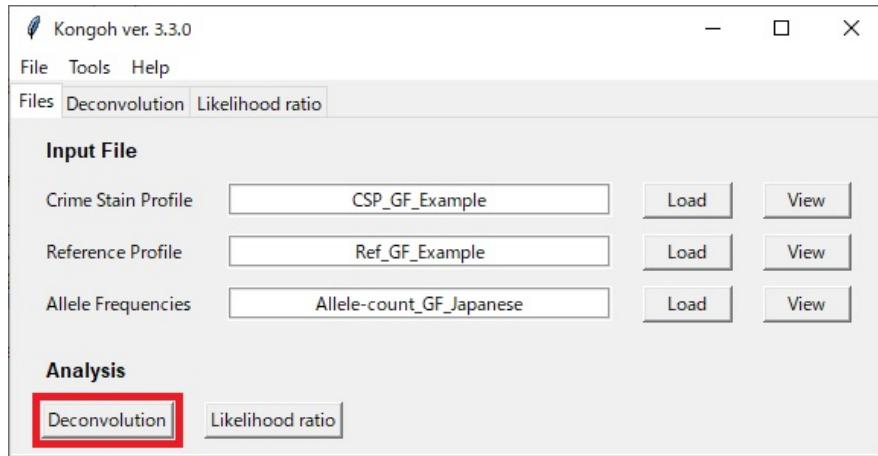
Figure 3: Format of the allele frequencies, which are represented as the allele counts

| Allele | D8S1179 | D21S11 | D7S820 | CSF1PO | D3S1358 | TH01 | D13S317 | D16S539 | D2S1338 | D19S433 | vWA | TPOX | D18S51 | D5S818 | FGA |
|--------|----------|--------|----------|----------|----------|----------|----------|----------|---------|----------|----------|----------|----------|----------|----------|
| 5 | | | | | | 0.001852 | | | | | | | | | |
| 6 | | | | | | 0.223621 | | | | | | | | | |
| 7 | 0.001852 | | 0.003332 | 0.010367 | | 0.266938 | 0.001852 | 0.001852 | | | 0.001852 | | 0.002962 | | |
| 8 | | | 0.12699 | 0.001852 | | 0.066642 | 0.267308 | 0.002221 | | | 0.454646 | | 0.007034 | | |
| 9 | 0.001852 | | 0.045909 | 0.04702 | | 0.398741 | 0.129211 | 0.353943 | | | 0.114402 | | 0.086264 | | |
| 9.1 | | | 0.001852 | | | | | | | | | | | | |
| 9.2 | | | | | | | | | | 0.001852 | | | | | |
| 9.3 | | | | | | 0.035172 | | | | | | | | | |
| 10 | 0.132914 | | 0.219178 | 0.223621 | | 0.009256 | 0.115143 | 0.196964 | | | | 0.363199 | 0.002592 | 0.201037 | |
| 10.1 | | | 0.001852 | | | | | | | | | | | | |
| 10.2 | | | | | | | | | | 0.001852 | | | | | |
| 10.3 | | | 0.001852 | | | | | | | | | | | | |
| 11 | 0.109219 | | 0.328767 | 0.208071 | | 0.221399 | 0.187338 | | | 0.004073 | | 0.363199 | 0.004813 | 0.292484 | |
| 11.2 | | | | | | | | | | 0.001852 | | | | | |
| 12 | 0.122917 | | 0.235098 | 0.418734 | 0.002221 | 0.202518 | 0.178823 | | | 0.040726 | | 0.035913 | 0.04813 | 0.235468 | |
| 12.2 | | | | | | | | | | 0.005553 | | | | | |
| 13 | 0.225102 | | 0.035172 | 0.069234 | 0.001852 | 0.051462 | 0.072936 | | | 0.287671 | 0.001852 | 0.001852 | 0.199556 | 0.166975 | |
| 13.2 | | | | | | | | | | 0.030359 | | | | | |
| 14 | 0.205109 | | 0.006294 | 0.018141 | 0.029248 | 0.013328 | 0.008515 | | | 0.34987 | 0.194372 | 0.001852 | 0.22251 | 0.009256 | |
| 14.2 | | | | | | | | | | 0.088486 | | | | | |
| 15 | 0.134765 | | 0.001852 | 0.005553 | 0.39615 | 0.001852 | 0.001852 | | | 0.051092 | 0.027027 | | 0.168456 | 0.001852 | |
| 15.2 | | | | | | | | | | 0.115143 | | | | | |
| 16 | 0.064421 | | 0.001852 | 0.306553 | | | | | | 0.008886 | 0.005553 | 0.184376 | | 0.125879 | |
| 16.2 | | | | | | | | | | 0.019622 | | | | | |
| 17 | 0.006664 | | | | 0.199926 | | | | | 0.097742 | | 0.282858 | | 0.081822 | 0.003702 |

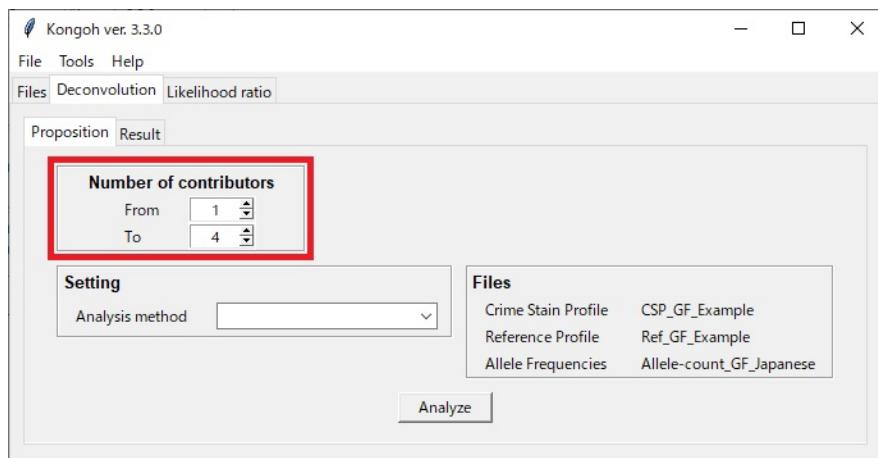
Figure 4: Format of the allele frequencies, which are represented as the allele probabilities

Deconvolution

1. Click the "Deconvolution" button in the "Files" tab.



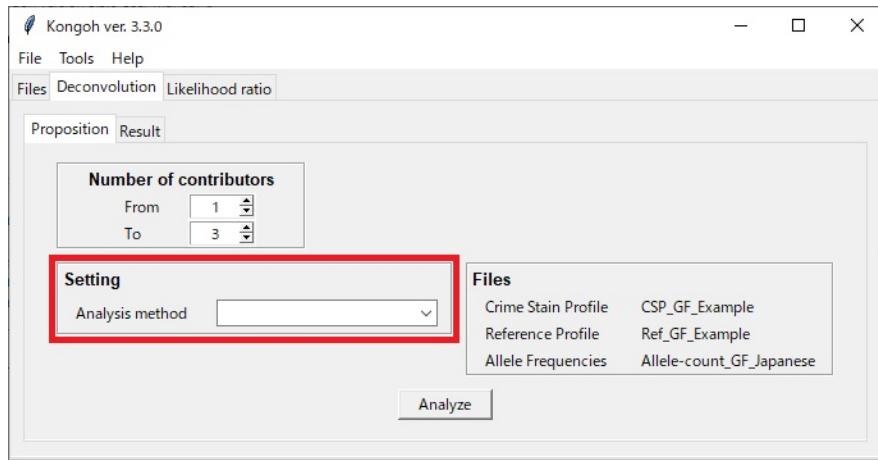
2. Set the range of the assumed number of contributors.



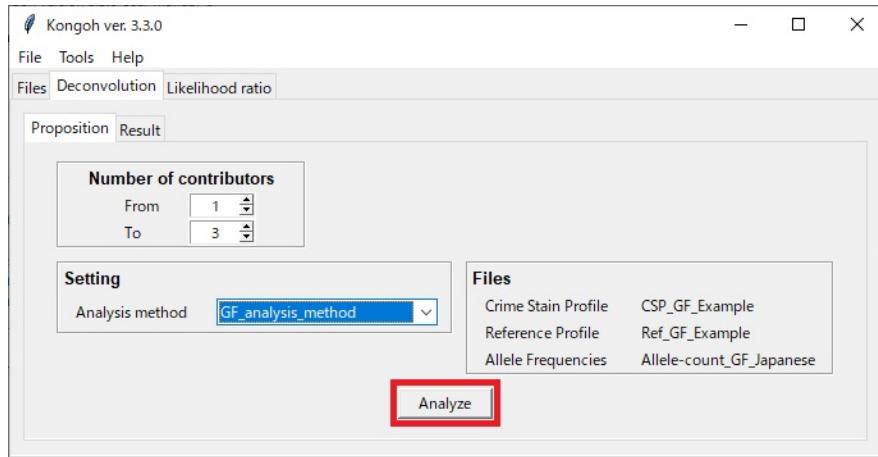
Note

The upper limit of the assumed number of contributors is four.

3. Select an analysis method².



4. Click the "Analyze" button.



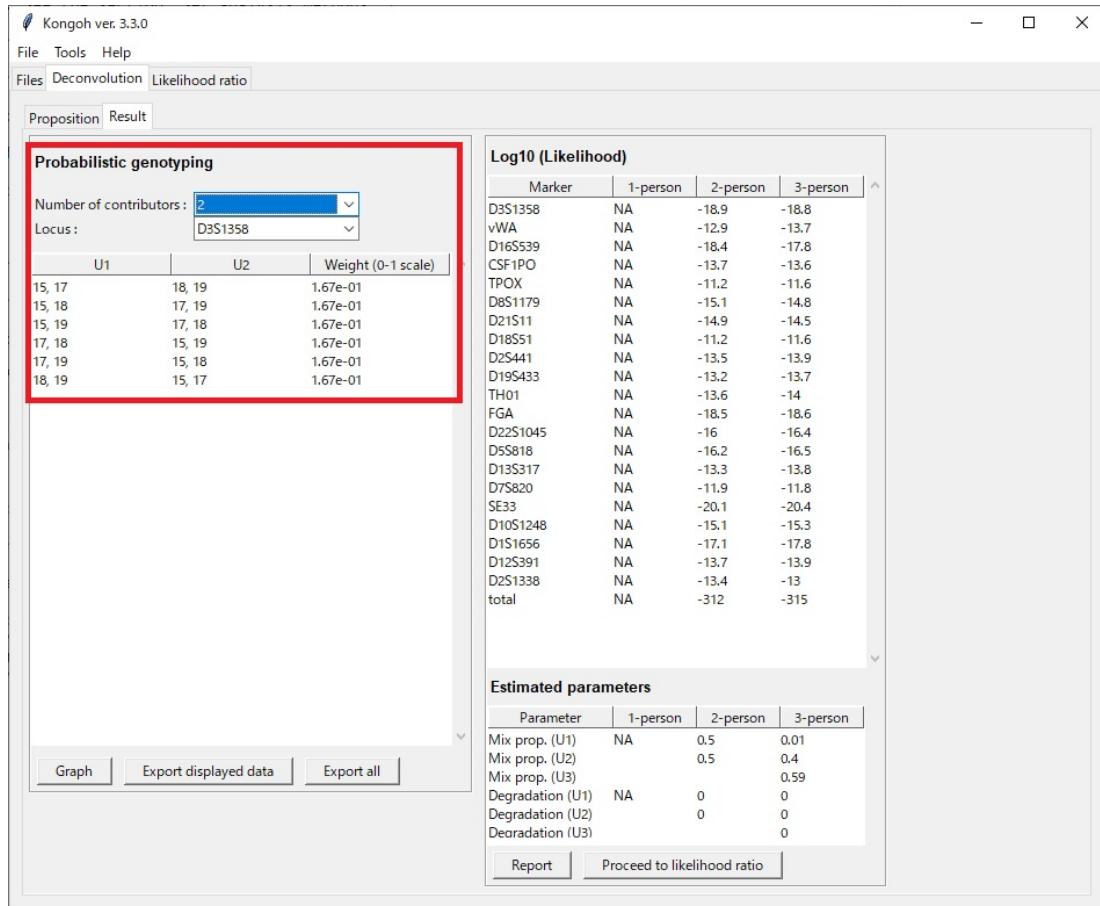
²Analysis methods can be set from Tools > Set analysis methods. See the section "Set analysis methods".

Then, the "Progress Bar" window will appear. After completing the analysis, the "Result" tab will appear automatically.

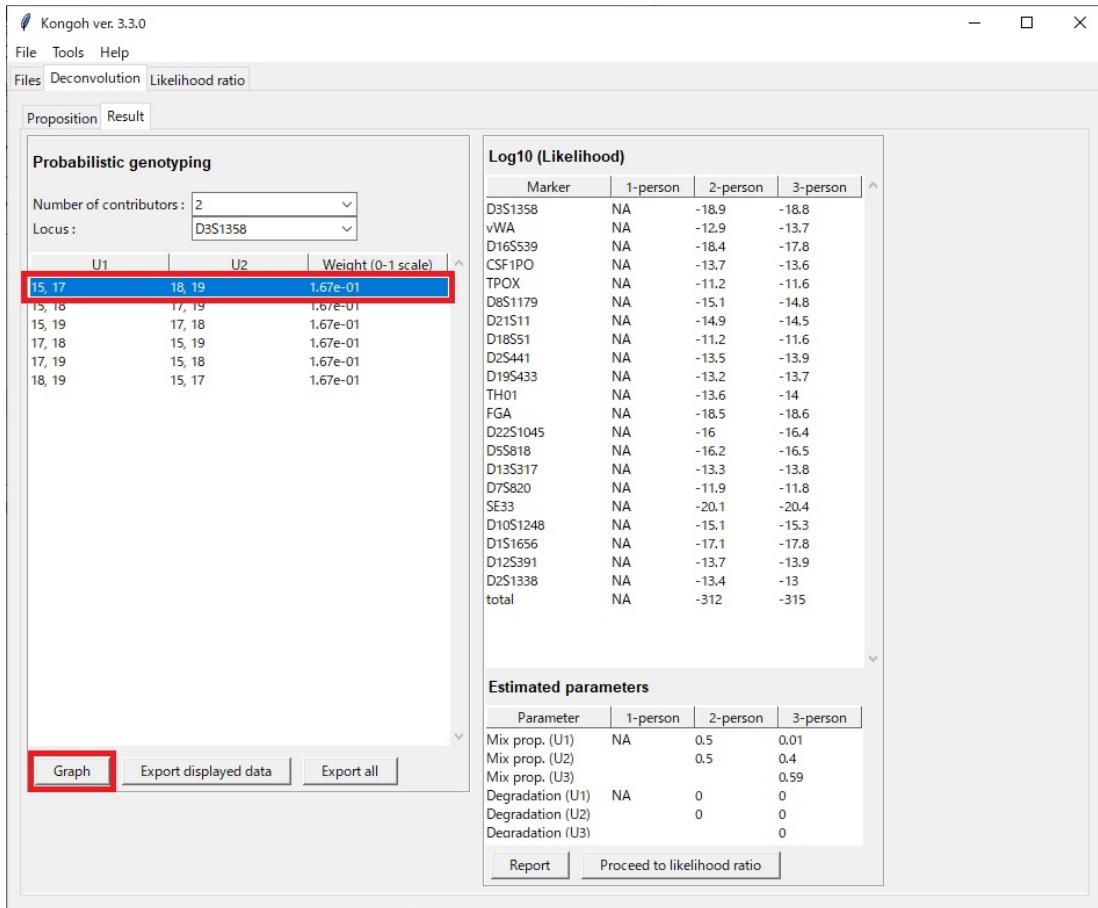


5. Review the result of probabilistic genotyping.

- The weight values of each genotype combination under the selected conditions are displayed.



- The observed peak heights and the expected peak heights of gamma distributions under the selected genotype combination can be compared by clicking the "Graph" button. An example of the graph is shown in Figure 5.



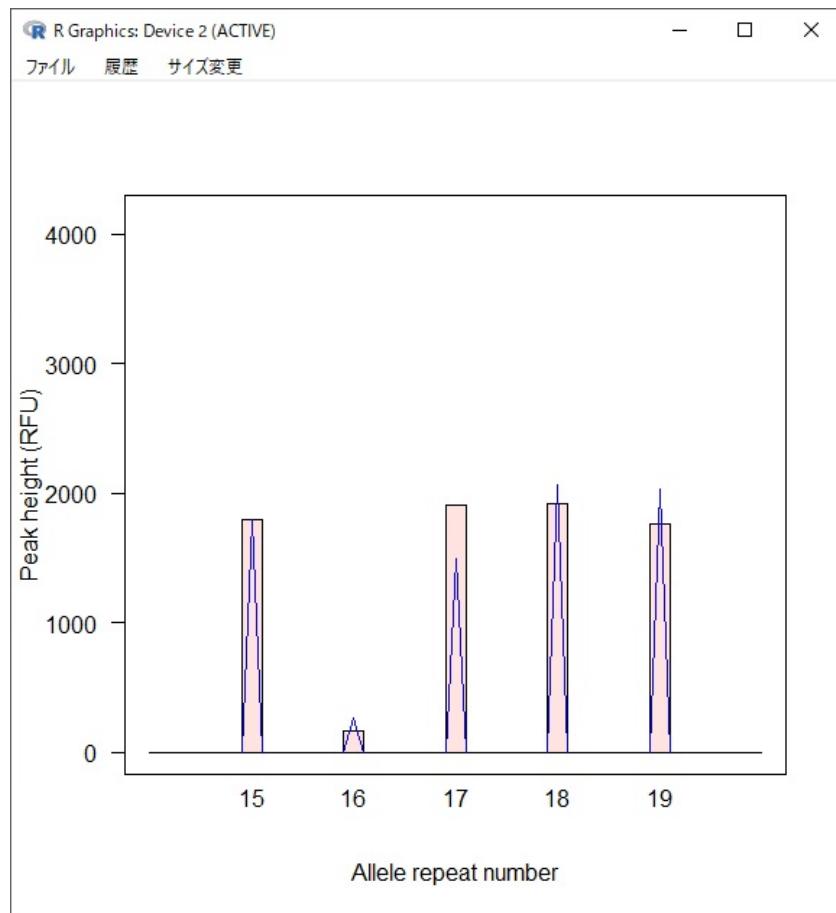
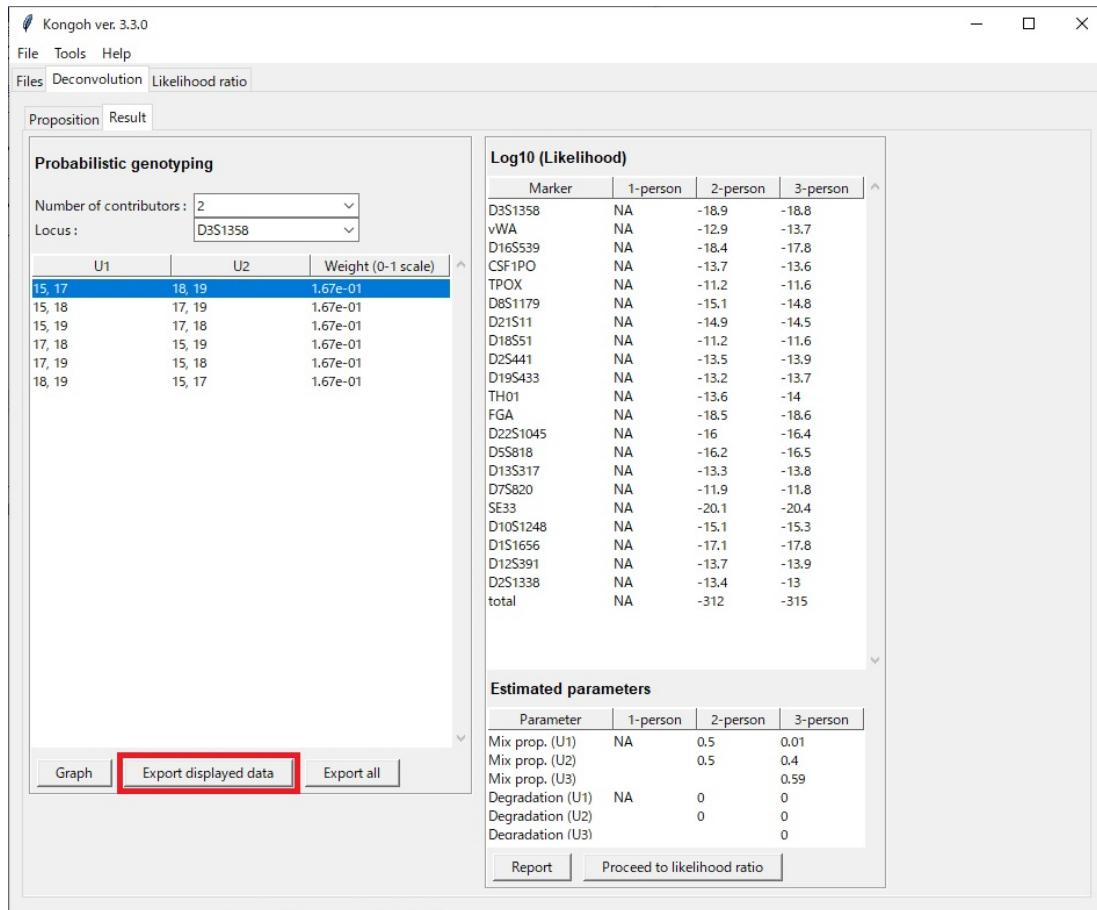
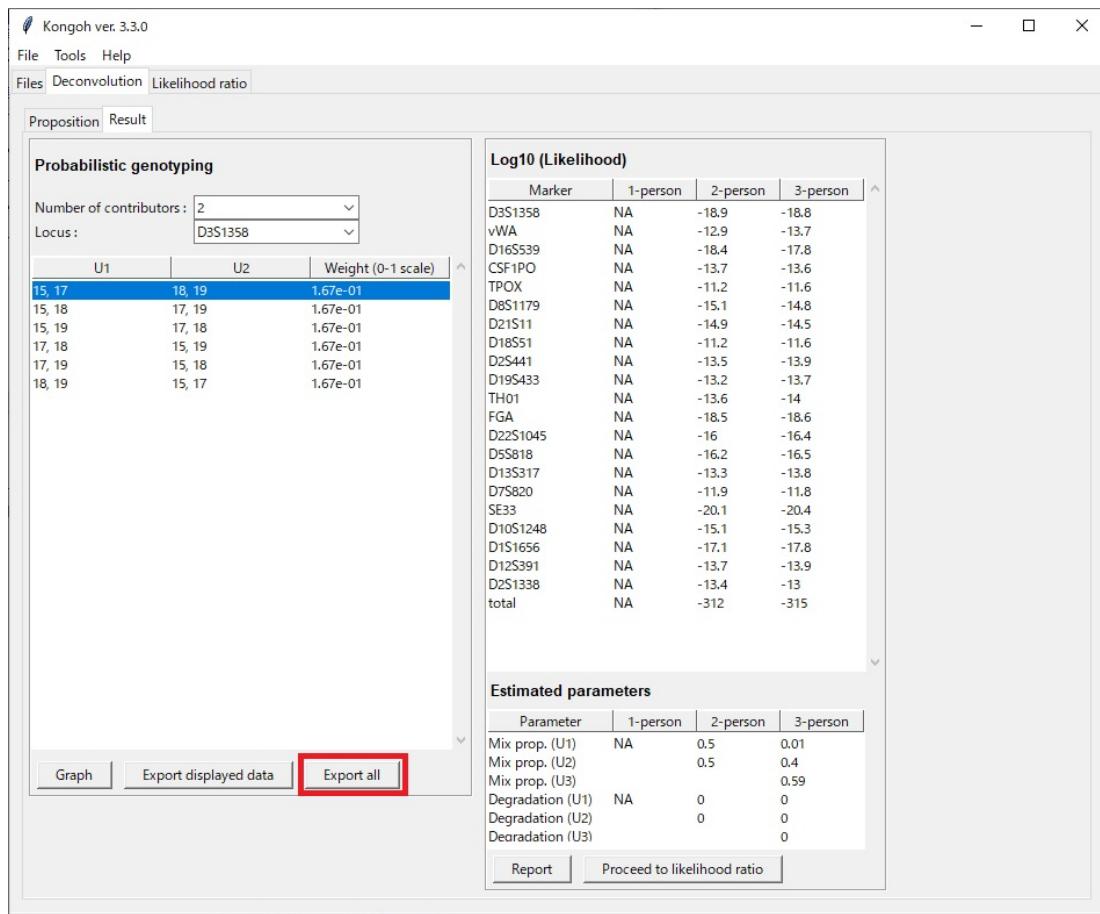


Figure 5: An example of the comparison between the observed peak heights (blue) and the expected peak heights (red)

- Displayed data can be exported by clicking the "Export displayed data" button.



- The results of all conditions can be exported by clicking the "Export all" button.



6. Review the result of the likelihoods and the estimated parameters.

- The report of probabilistic genotyping can be exported by clicking the "Report" button.

Probabilistic genotyping

| U1 | U2 | Weight (0-1 scale) |
|--------|--------|--------------------|
| 15, 17 | 18, 19 | 1.67e-01 |
| 15, 18 | 17, 19 | 1.67e-01 |
| 15, 19 | 17, 18 | 1.67e-01 |
| 17, 18 | 15, 19 | 1.67e-01 |
| 17, 19 | 15, 18 | 1.67e-01 |
| 18, 19 | 15, 17 | 1.67e-01 |

Log10 (Likelihood)

| Marker | 1-person | 2-person | 3-person |
|----------|----------|----------|----------|
| D3S1358 | NA | -18.9 | -18.8 |
| vWA | NA | -12.9 | -13.7 |
| D16S539 | NA | -18.4 | -17.8 |
| CSF1PO | NA | -13.7 | -13.6 |
| TPOX | NA | -11.2 | -11.6 |
| D8S1179 | NA | -15.1 | -14.8 |
| D21S11 | NA | -14.9 | -14.5 |
| D18S51 | NA | -11.2 | -11.6 |
| D2S441 | NA | -13.5 | -13.9 |
| D19S433 | NA | -13.2 | -13.7 |
| TH01 | NA | -13.6 | -14 |
| FGA | NA | -18.5 | -18.6 |
| D22S1045 | NA | -16 | -16.4 |
| D5S818 | NA | -16.2 | -16.5 |
| D13S317 | NA | -13.3 | -13.8 |
| D7S820 | NA | -11.9 | -11.8 |
| SE33 | NA | -20.1 | -20.4 |
| D10S1248 | NA | -15.1 | -15.3 |
| D1S1656 | NA | -17.1 | -17.8 |
| D12S391 | NA | -13.7 | -13.9 |
| D2S1338 | NA | -13.4 | -13 |
| total | NA | -312 | -315 |

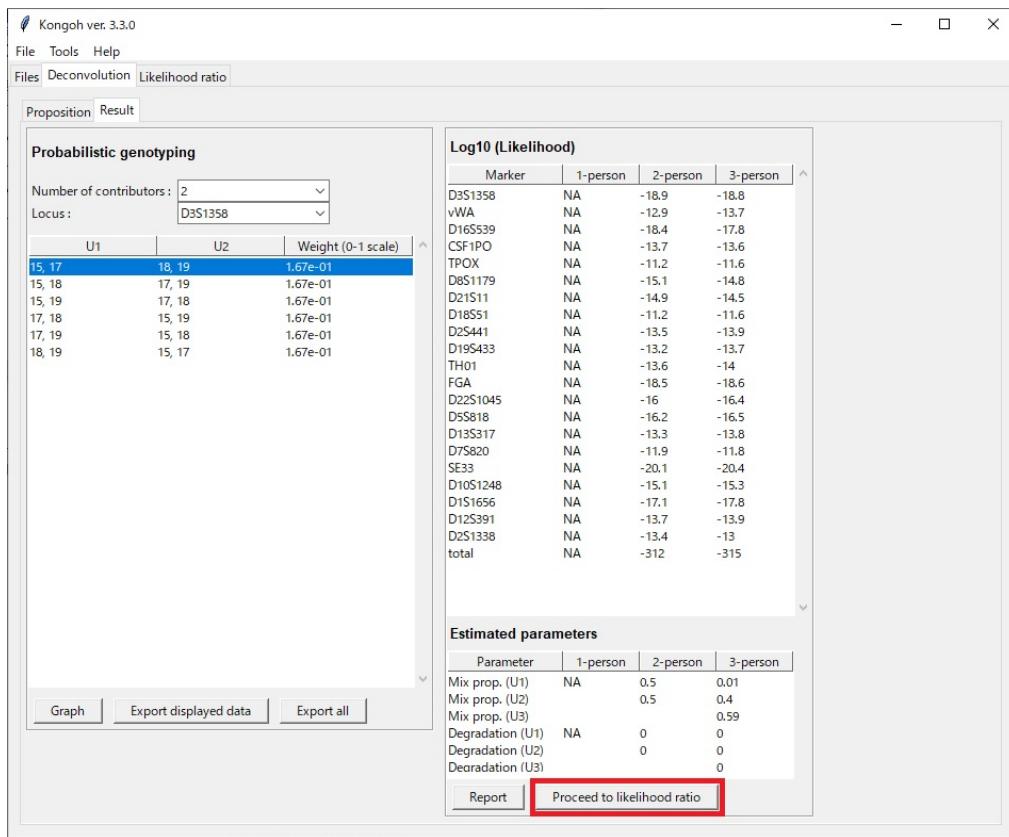
Estimated parameters

| Parameter | 1-person | 2-person | 3-person |
|------------------|----------|----------|----------|
| Mix prop. (U1) | NA | 0.5 | 0.01 |
| Mix prop. (U2) | | 0.5 | 0.4 |
| Mix prop. (U3) | | | 0.59 |
| Degradation (U1) | NA | 0 | 0 |
| Degradation (U2) | | 0 | 0 |
| Degradation (U3) | | | 0 |

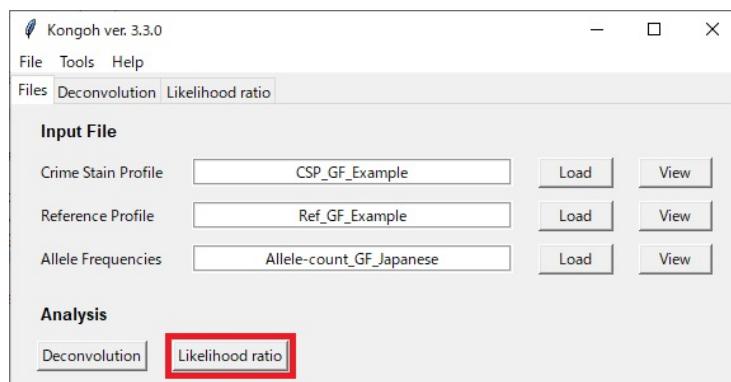
Report **Proceed to likelihood ratio**

Likelihood ratio

1. Click the "Proceed to likelihood ratio" button in the "Result" tab for "Deconvolution".



Or click the "Likelihood ratio" button in the "Files" tab.



2. Set both the prosecutor (Hp) and defense (Hd) hypotheses. Check the individuals to include them as contributors in each hypothesis.

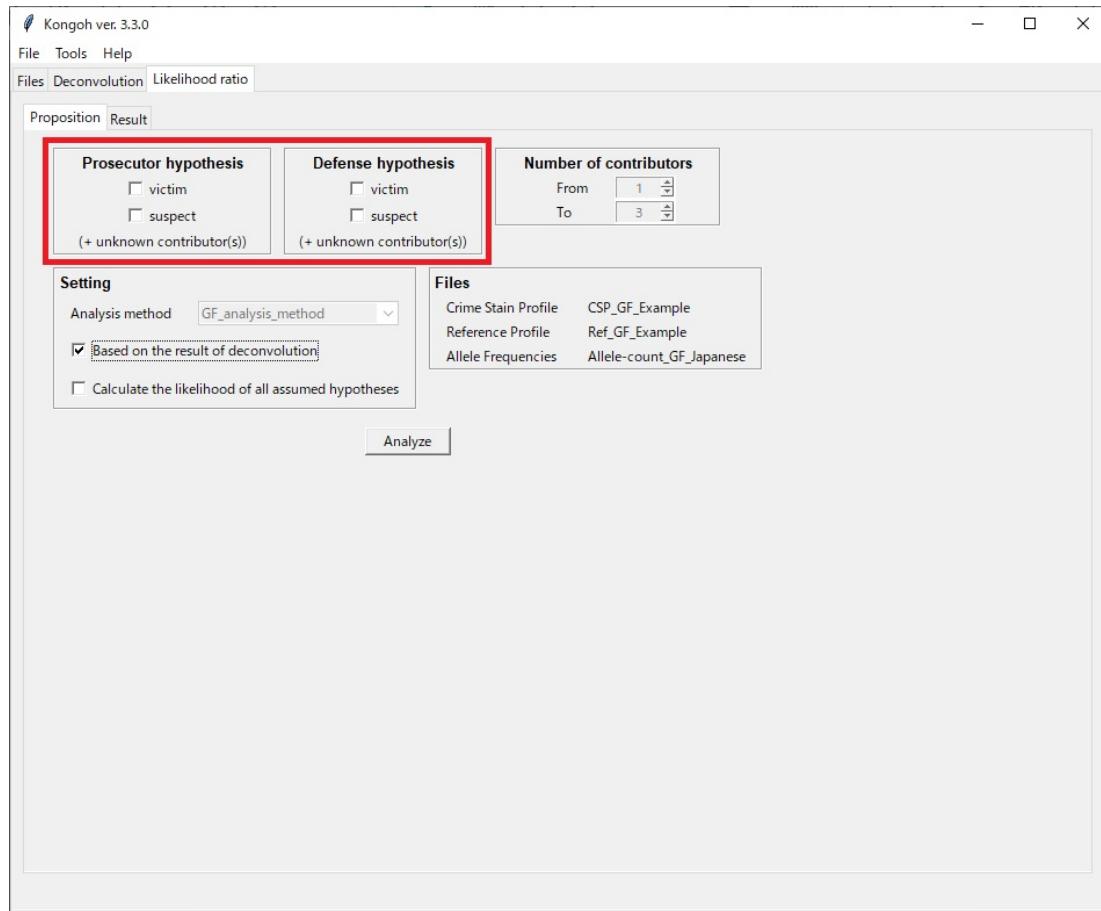


Fig. 6 shows an example of setting the hypotheses:

- Hp: victim + suspect (+ unknown contributor(s))
- Hd: victim (+ unknown contributor(s))

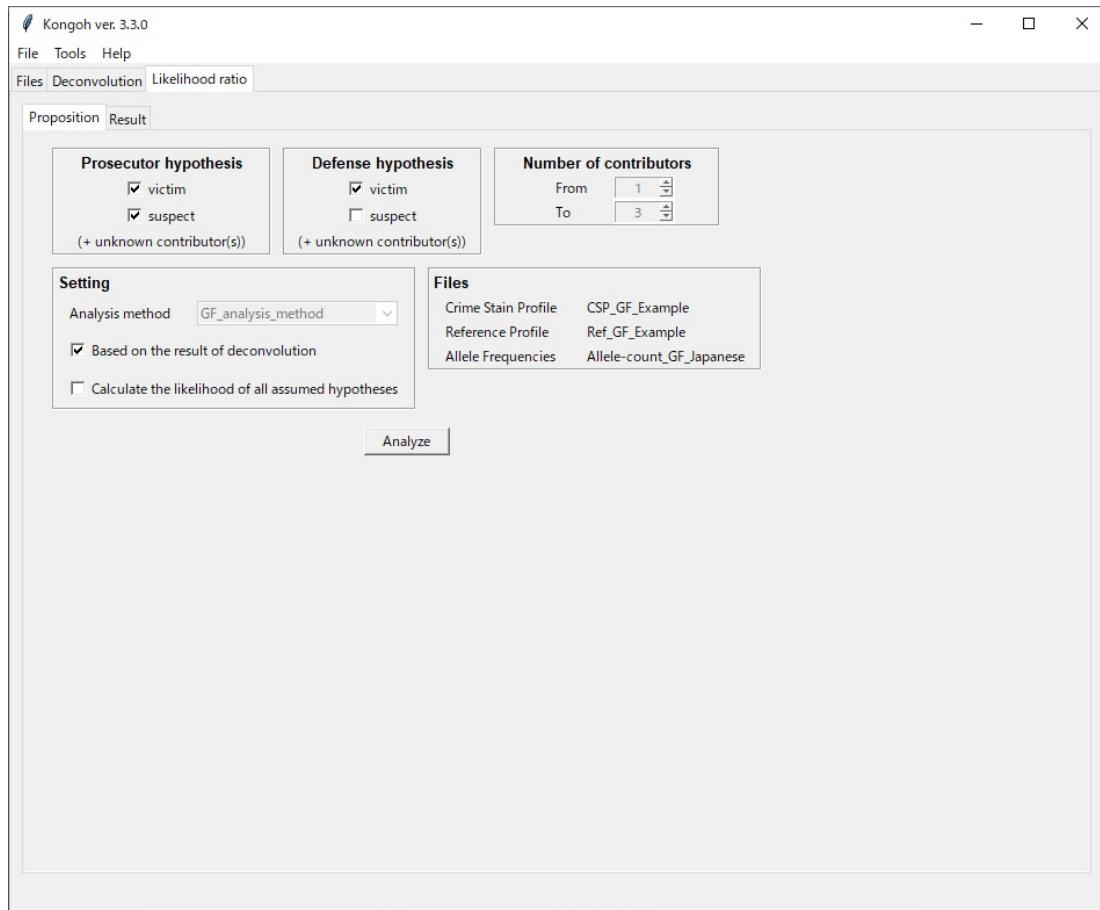
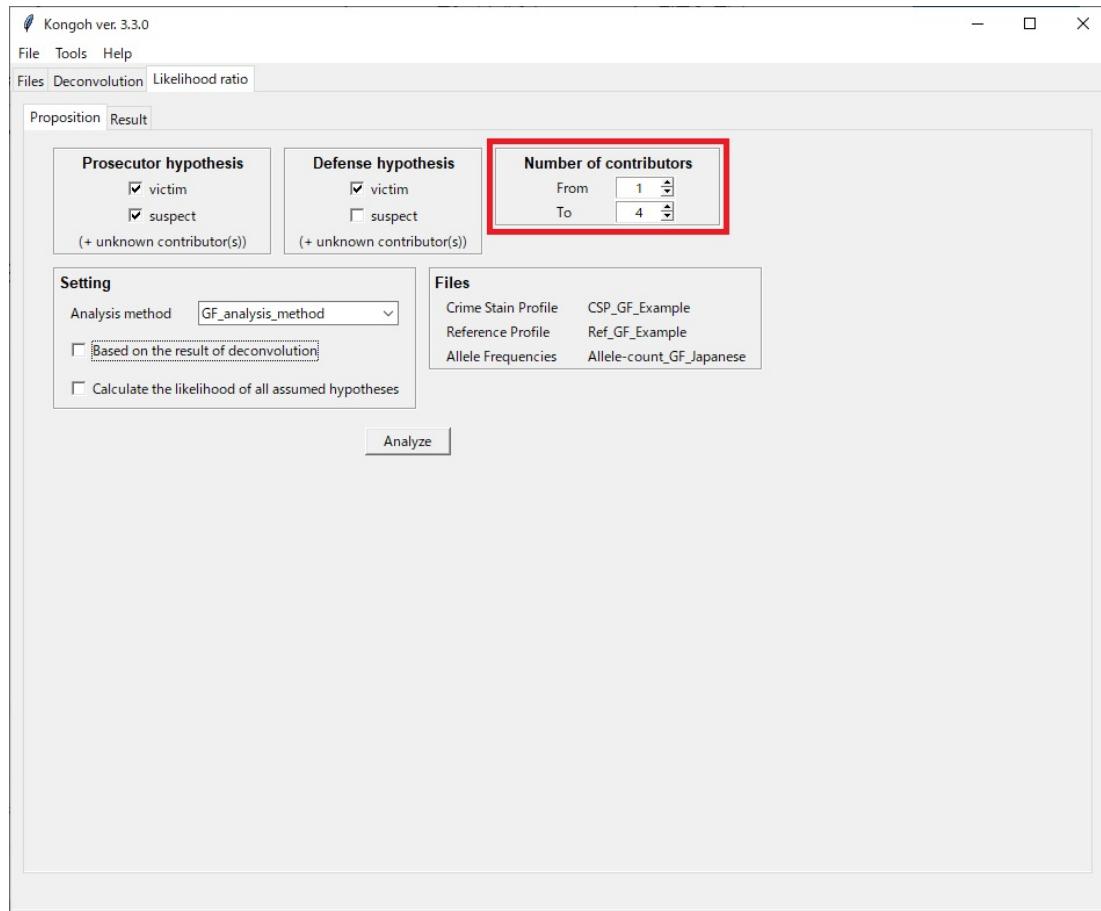


Figure 6: An example of setting the hypotheses

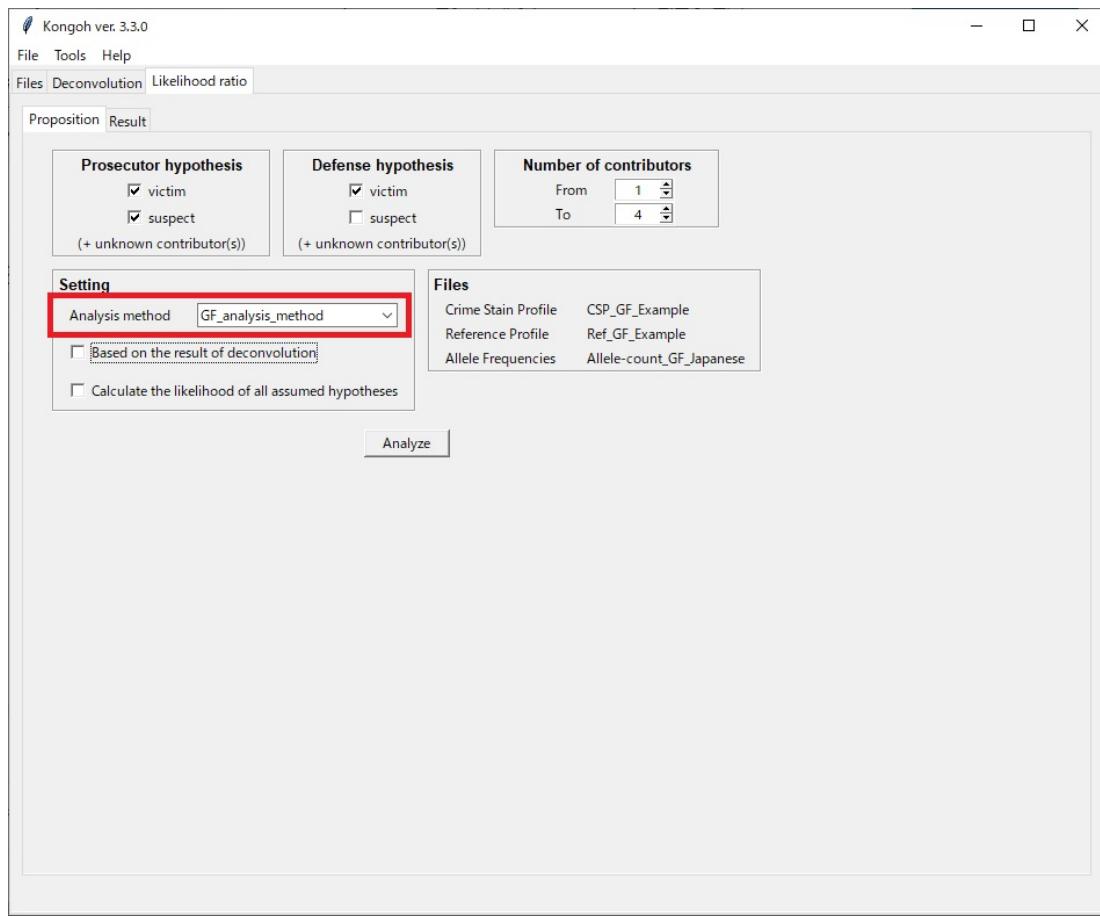
3. Set the range of the assumed number of contributors. The likelihoods of all set numbers are calculated for both Hp and Hd.



Note

- If the "Based on the result of deconvolution" is checked, the range of the assumed number of contributors cannot be changed.
- The upper limit of the assumed number of contributors is four.

4. Select an analysis method³.

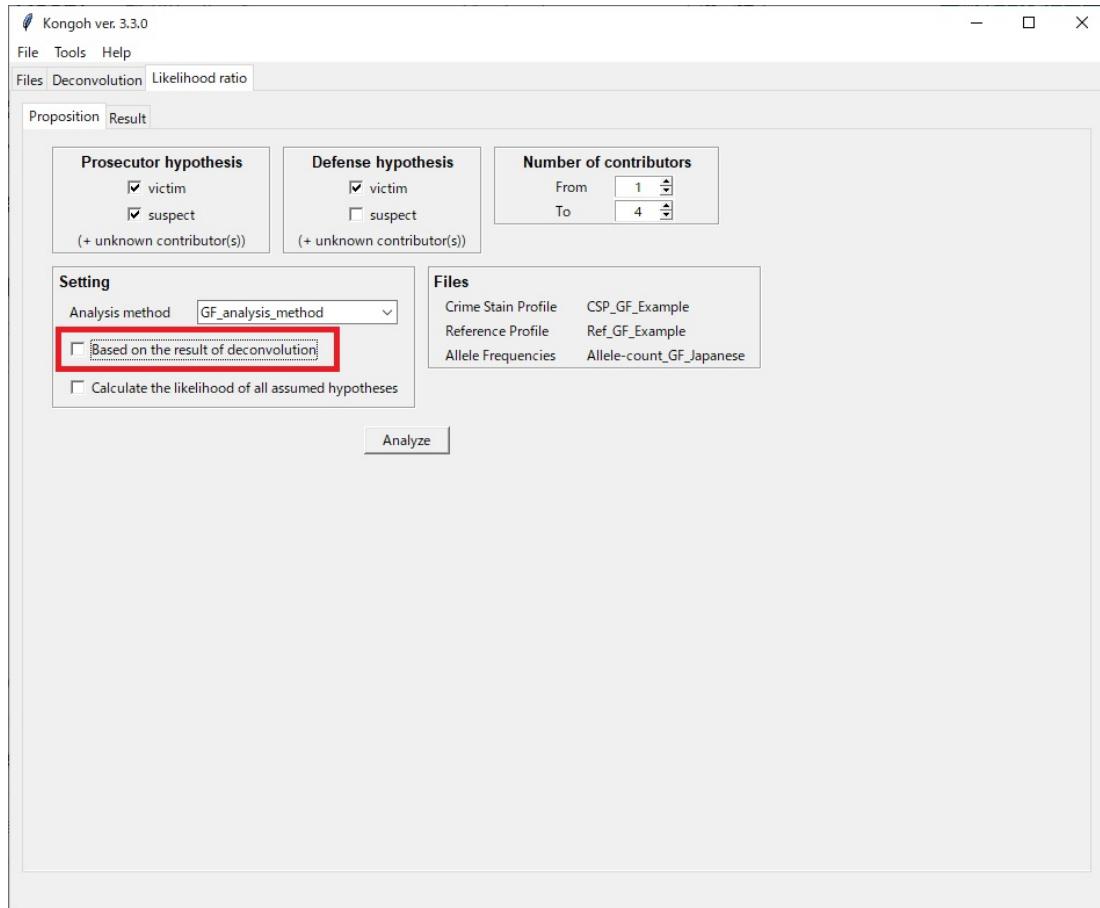


Note

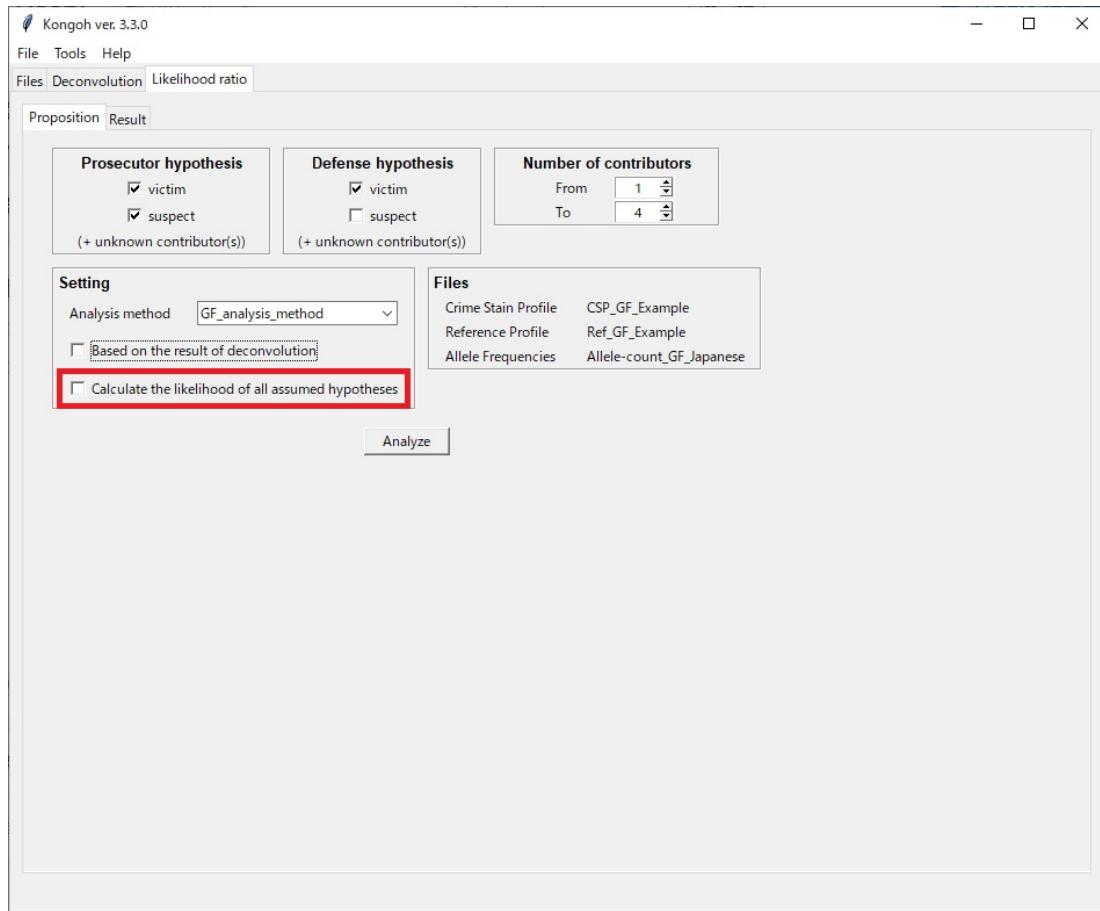
- If the "Based on the result of deconvolution" is checked, the analysis method cannot be changed.

³Analysis methods can be set from Tools > Set analysis methods. See the section "Set analysis methods".

5. To calculate the likelihood ratio based on the result of deconvolution, check the "Based on the result of deconvolution".



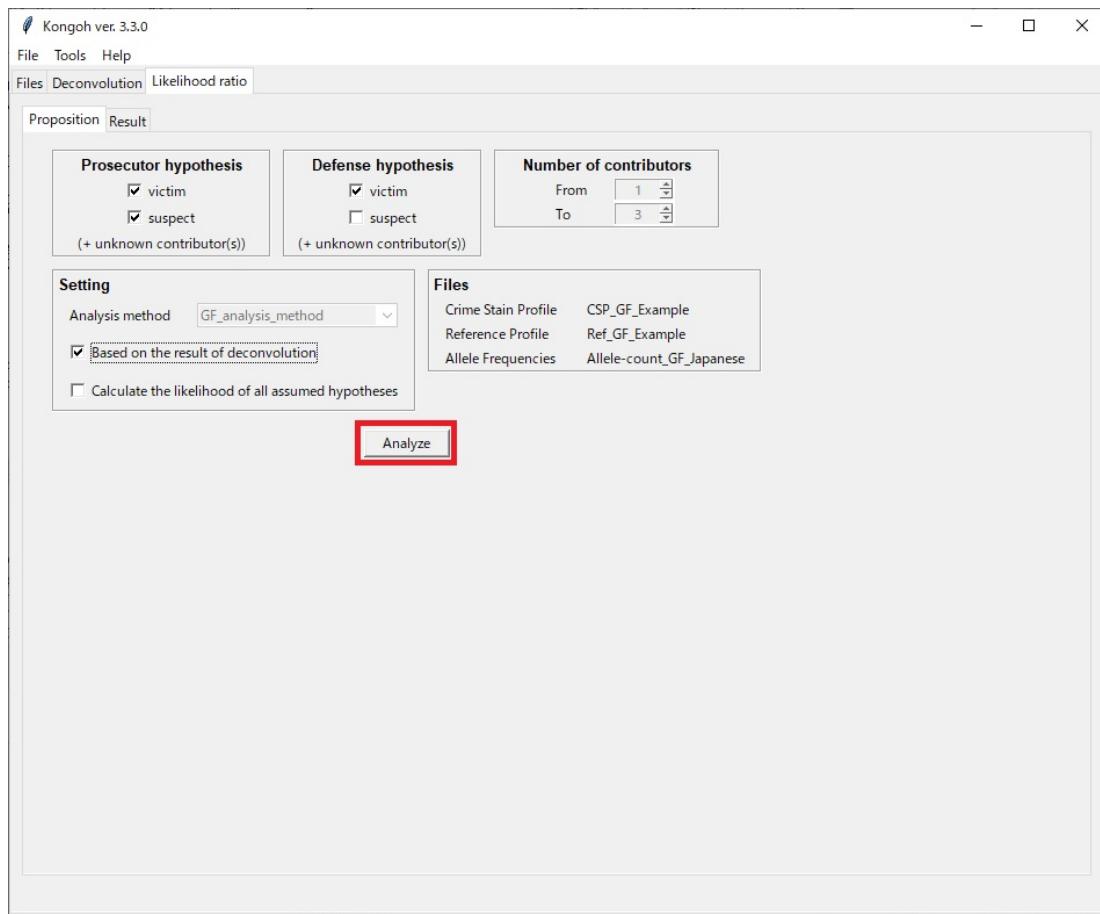
6. The likelihoods of all assumed hypotheses can be calculated by checking the "Calculate the likelihood of all assumed hypotheses".



For example, when the reference profiles of the victim and the suspect are inputted and the range of the assumed number of contributors is 2 to 3, all assumed hypotheses are as follows:

- two unknown contributors
- victim + one unknown contributor
- suspect + one unknown contributor
- victim + suspect
- three unknown contributors
- victim + two unknown contributors
- suspect + two unknown contributors
- victim + suspect + one unknown contributor

7. Click the "Analyze" button.

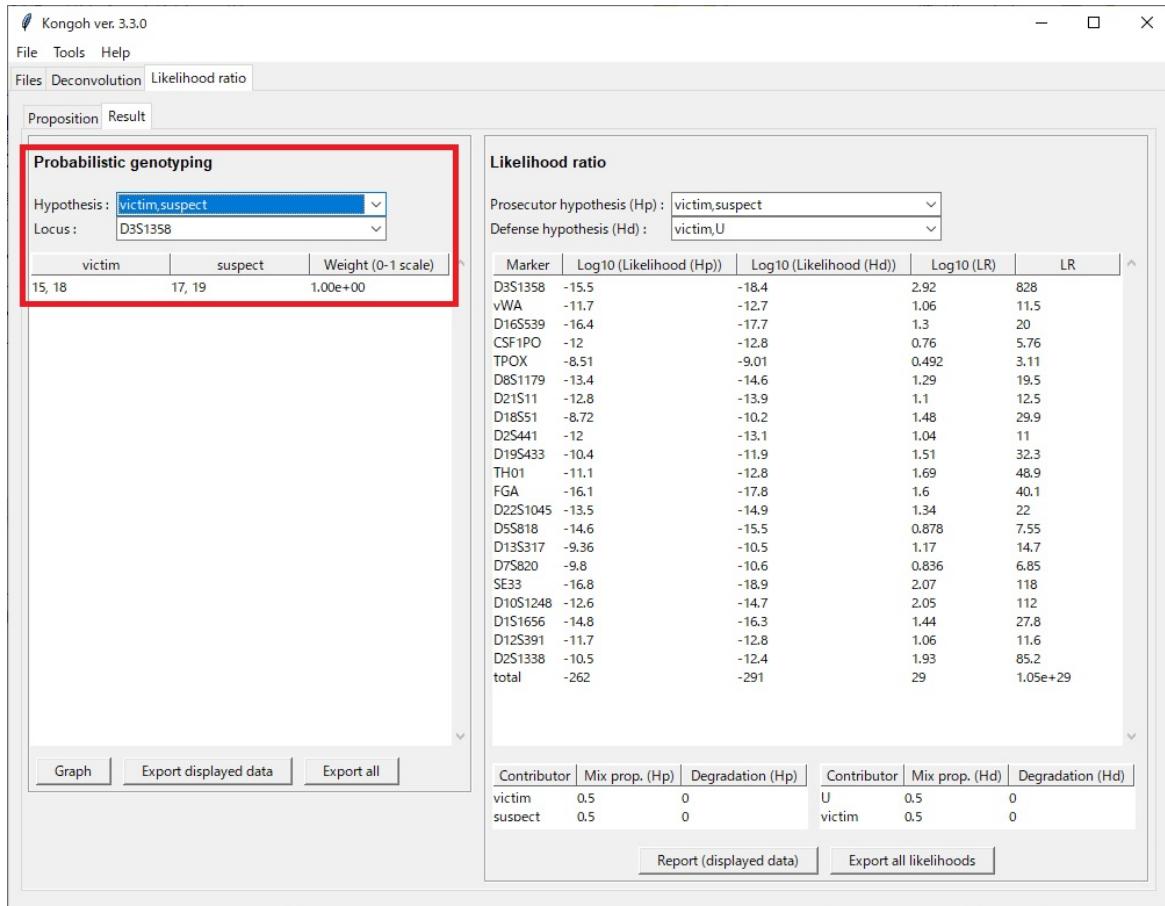


Then, the "Progress Bar" window will appear. After completing the analysis, the "Result" tab will appear automatically.

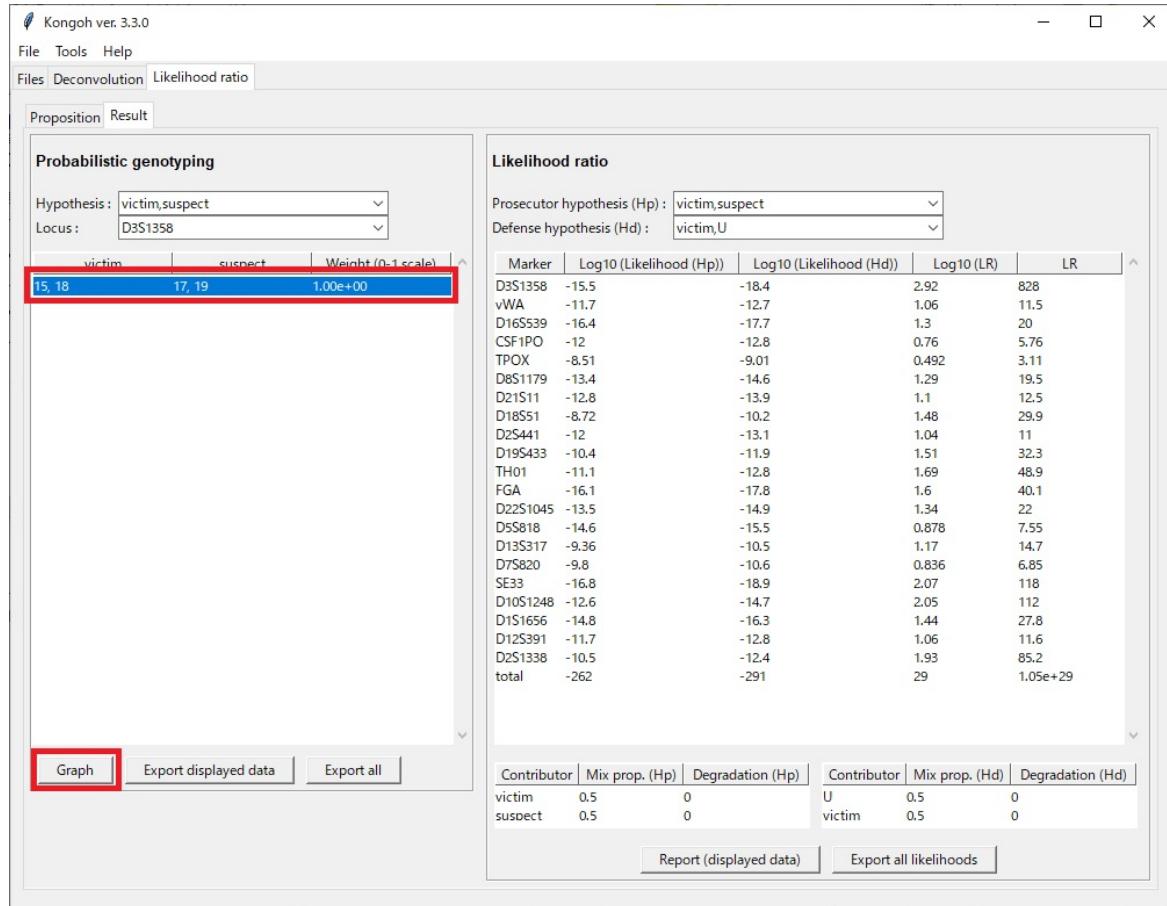


8. Review the result of probabilistic genotyping.

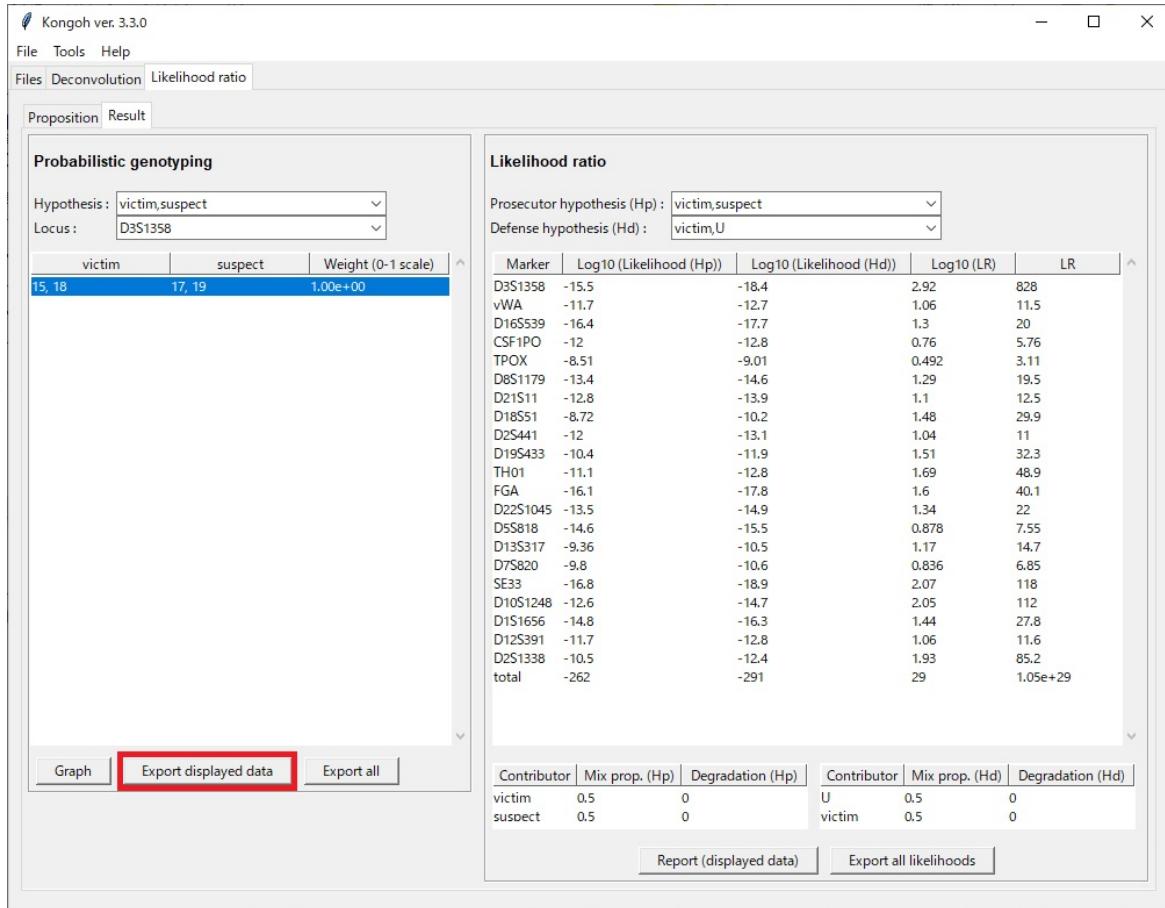
- The weight values of each genotype combination under the selected conditions are displayed.



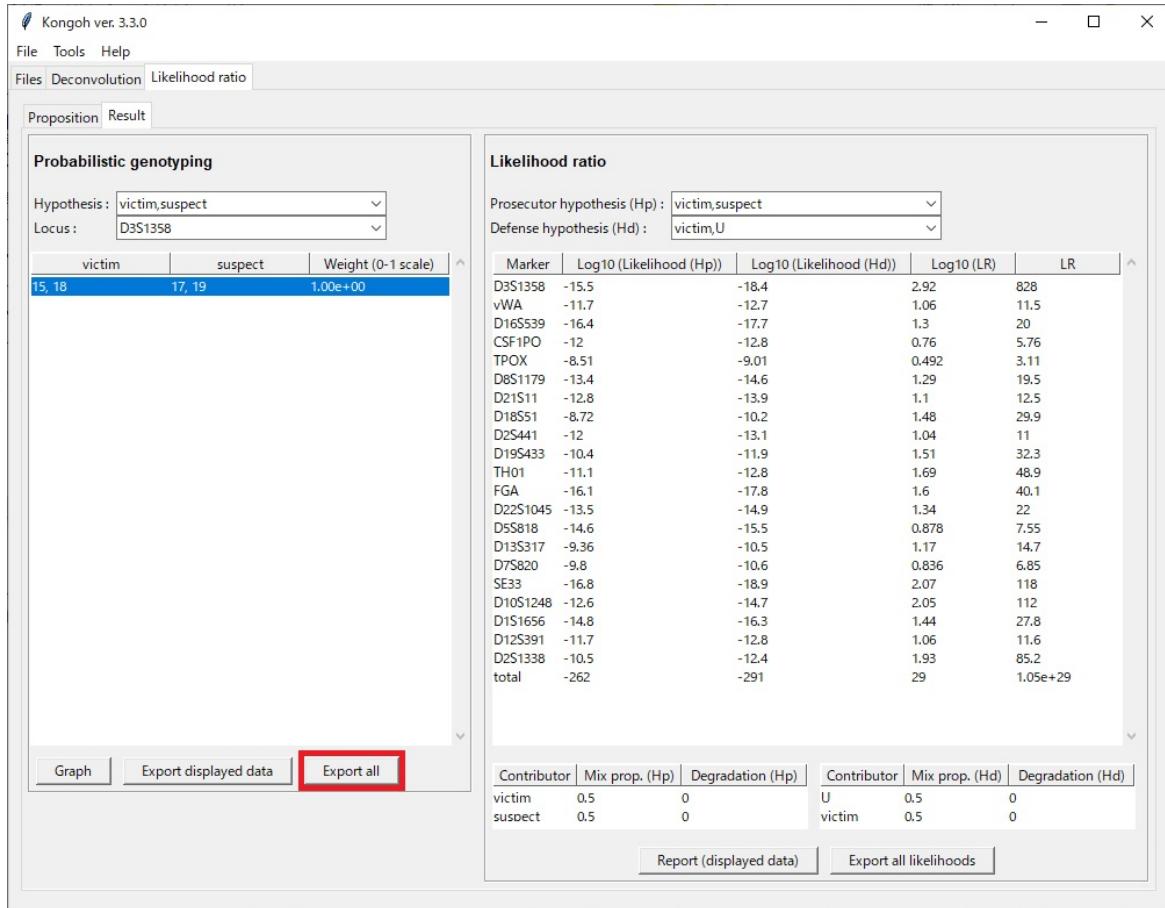
- The observed peak heights and the expected peak heights of gamma distributions under the selected genotype combination can be compared by clicking the "Graph" button.



- Displayed data can be exported by clicking the "Export displayed data" button.

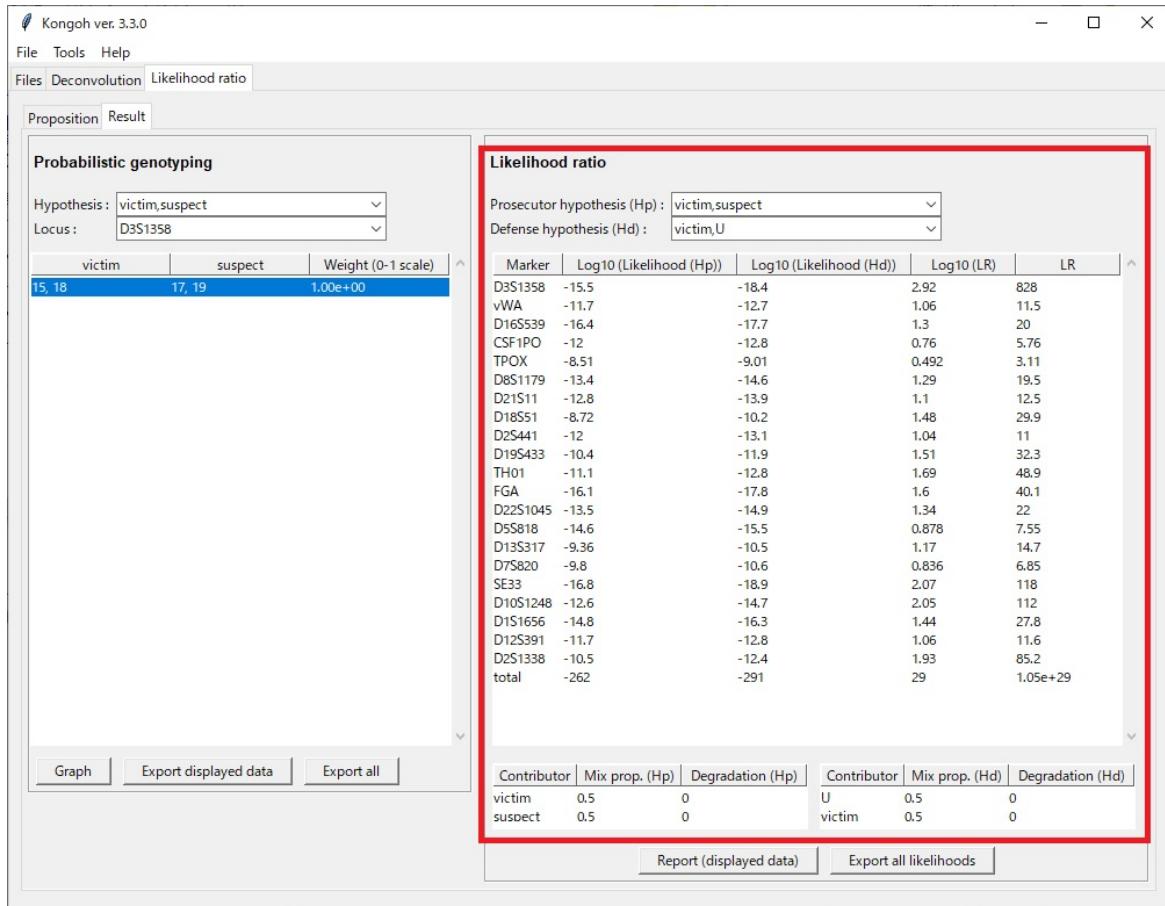


- The results of all conditions can be exported by clicking the "Export all" button.

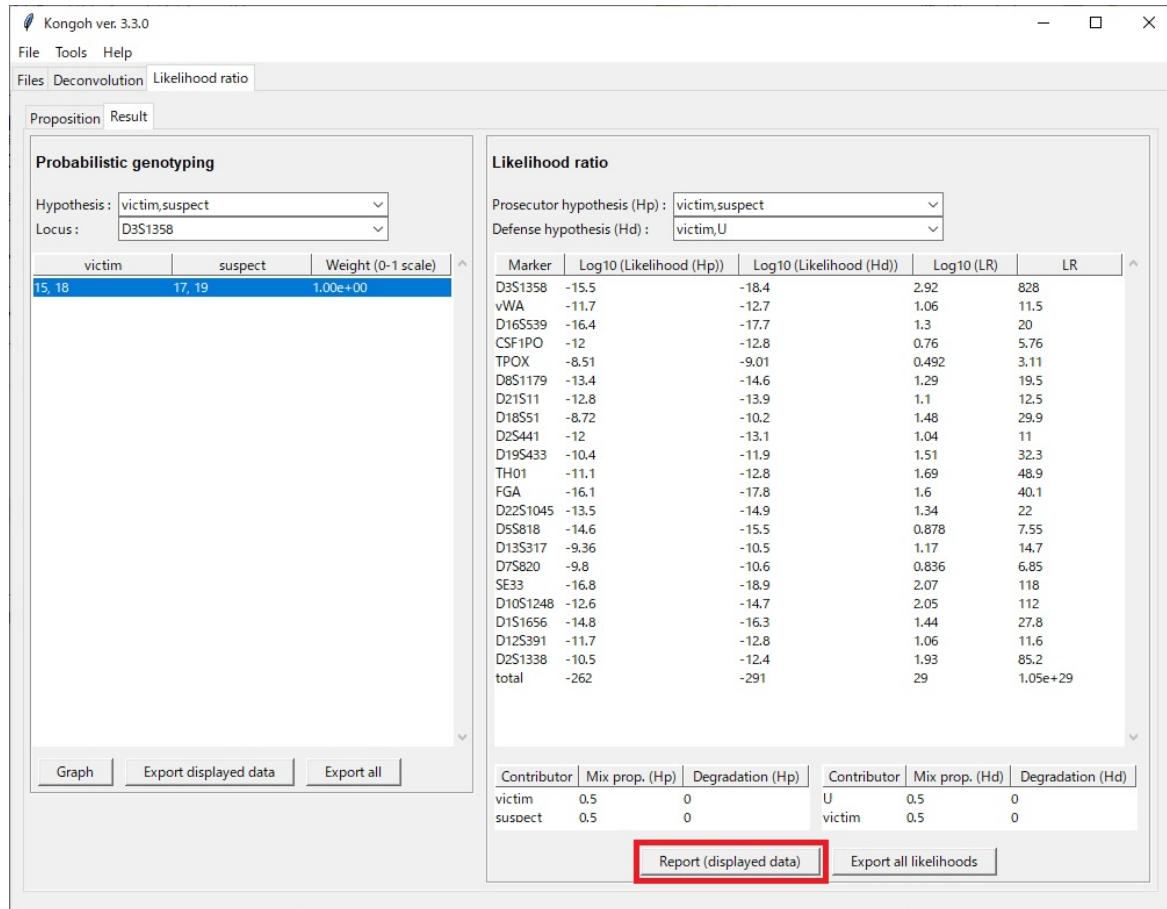


9. Review the result of the likelihood ratio.

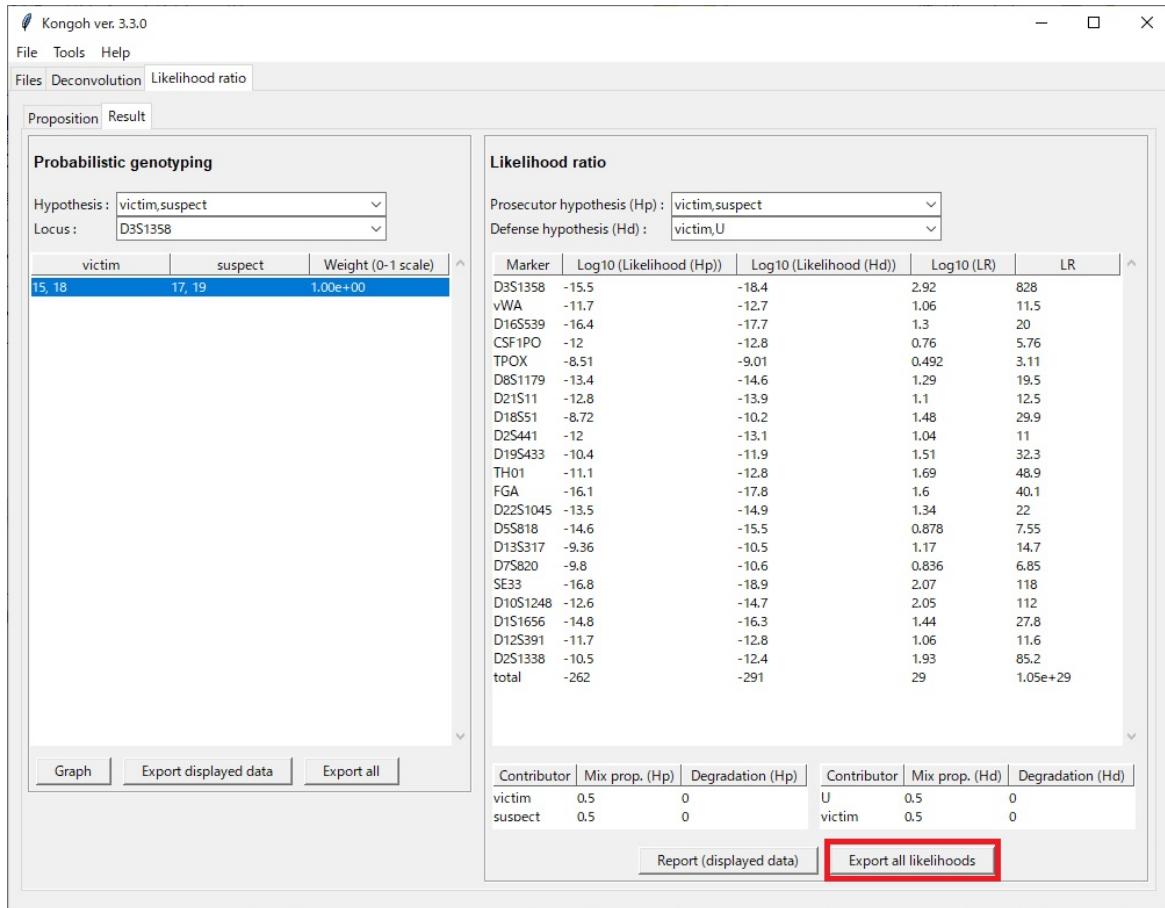
- The likelihood ratio, the estimated mixture proportion, and the estimated degradation parameter under the selected hypotheses are displayed.



- The report of the likelihood ratio for displayed data can be exported by clicking the "Report (displayed data)" button.



- Likelihoods and LRs of all assumed hypotheses can be exported by clicking the "Export all likelihoods" button.

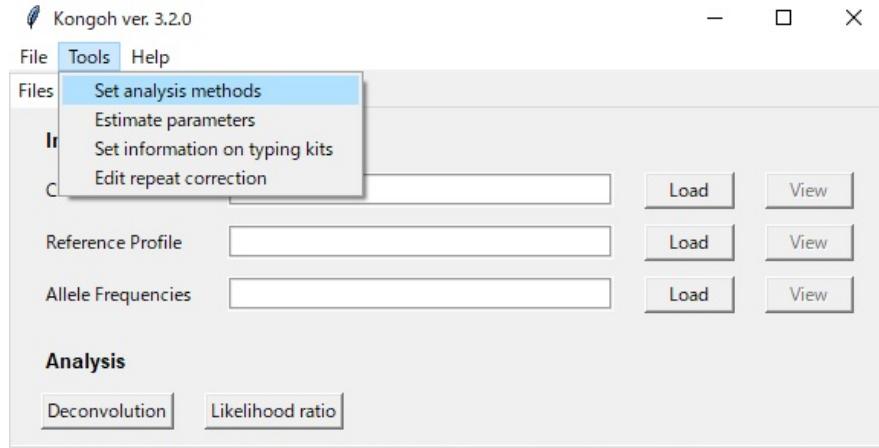


Set analysis methods

Since ver. 3.2.0, users must create an analysis method to determine the conditions for performing deconvolution and assigning likelihood ratios. This section describes how to create and edit an analysis method.

Create an analysis method

1. Go to Tools > Set analysis methods.

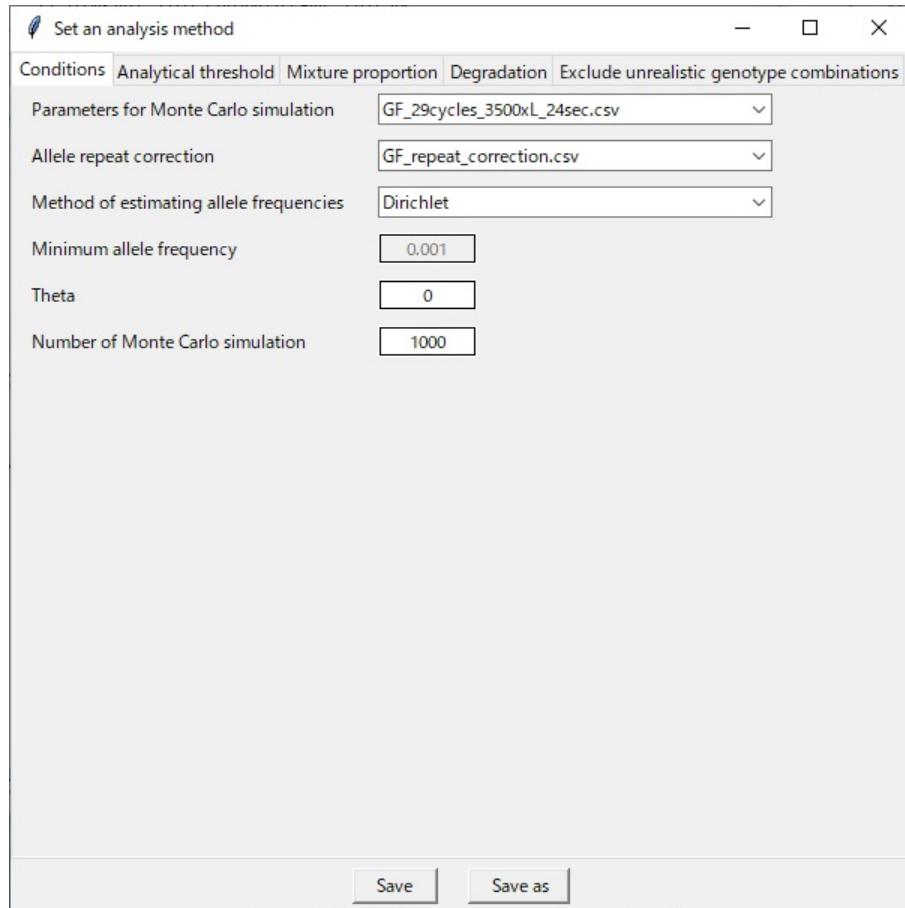


2. Click the "New" button to create an analysis method.



3. Enter or select the settings shown in the figures on the following pages.

”Conditions” tab



Parameters for Monte Carlo simulation

Select a file of the parameters for Monte Carlo simulation. The file can be generated in the function of “Estimate parameters”⁴. The default files for Identifier Plus (IDP_28cycles_3130xl_10sec.csv)⁵ and GlobalFiler (GF_29cycles_3500xL_24sec.csv)⁶ are provided in the package *Kongoh*.

⁴See the section ”Estimate parameters”.

⁵Default parameters of Identifier Plus were estimated using 392 single-source profiles, which were obtained from publicly available datasets in the Project Research Openness for Validation with Empirical Data (PROVEDIt: <https://lftdi.camden.rutgers.edu/provedit/files/>). These profiles were derived from non-degraded DNA of 50 individuals. The DNA amount of each sample was 0.0078–0.73 ng. The DNA samples were amplified at 28 cycles, and PCR products were analyzed on an Applied Biosystems™ 3130xl Genetic Analyzer (Thermo Fisher Scientific) with injection parameters set at 3 kV for 10 s.

⁶Default parameters of GlobalFiler were estimated using 300 single-source profiles. These profiles were derived from pristine DNA of 50 individuals. The extracted DNA was diluted to 0.1, 0.05, 0.025, 0.0125, 0.00625, and 0.003125 ng/ L. A total of 300 diluted DNA solutions were amplified in 29 cycles using the GlobalFiler kit. Subsequently, PCR products were analyzed on an Applied Biosystems™ 3500xL Genetic Analyzer (Thermo Fisher Scientific) with injection parameters set at 1.2 kV for 24 s in the Data Collection Software v3.1 (Thermo Fisher Scientific). See Manabe et al., 2021[5].

Allele repeat correction

Select a file of the allele repeat correction. The file can be generated in the function of "Estimate parameters". The default files for Identifier Plus (IDP_repeat_correction.csv) and GlobalFiler (GF_repeat_correction.csv) are provided in the package *Kongoh*.

Method of estimating allele frequencies

If the allele frequencies of input files are represented as the allele counts, select "Dirichlet". If the allele frequencies of input files are represented as the allele probabilities, select "No correction".

Minimum allele frequency

The minimum allele frequency is applied to the frequency of the unobserved alleles when the "Method of estimating allele frequencies" is set to "No correction".

Theta

The theta value is used to consider the subpopulation effect.

Number of Monte Carlo simulation

The default value is 1,000, which is considered sufficient for simulations based on developmental validation. If the number of simulations is increased, then the result becomes more robust; however, the runtime increases as well.

"Analytical threshold" tab

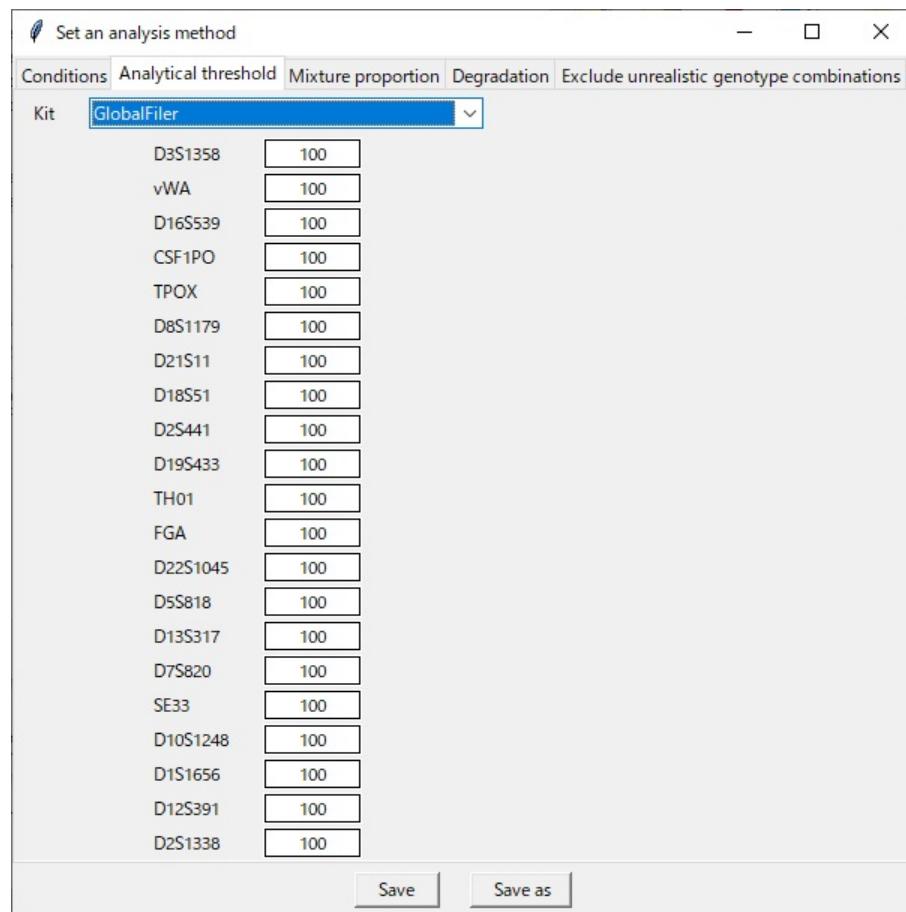
Set an analysis method

Conditions Analytical threshold Mixture proportion Degradation Exclude unrealistic genotype combinations

Kit GlobalFiler

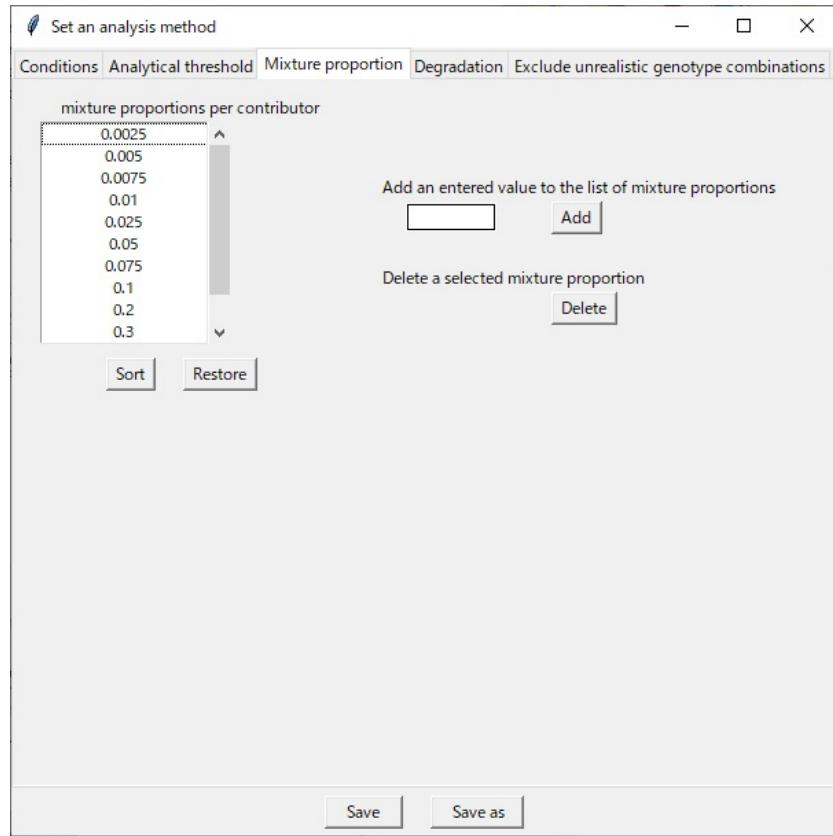
| | |
|----------|-----|
| D3S1358 | 100 |
| vWA | 100 |
| D16S539 | 100 |
| CSF1PO | 100 |
| TPOX | 100 |
| D8S1179 | 100 |
| D21S11 | 100 |
| D18S51 | 100 |
| D2S441 | 100 |
| D19S433 | 100 |
| TH01 | 100 |
| FGA | 100 |
| D22S1045 | 100 |
| D5S818 | 100 |
| D13S317 | 100 |
| D7S820 | 100 |
| SE33 | 100 |
| D10S1248 | 100 |
| D1S1656 | 100 |
| D12S391 | 100 |
| D2S1338 | 100 |

Save Save as



Select a kit, then enter the analytical thresholds of each locus.

"Mixture proportion" tab



Users can manually add or delete the mixture proportion per contributor. MP_n is the DNA proportion of the contributor $n (n = 1, 2, \dots, N)$ in a crime stain profile, where N is the number of contributors. In Kongoh, MP_n must satisfy the following conditions:

$$\sum_{n=1}^N MP_n = 1,$$

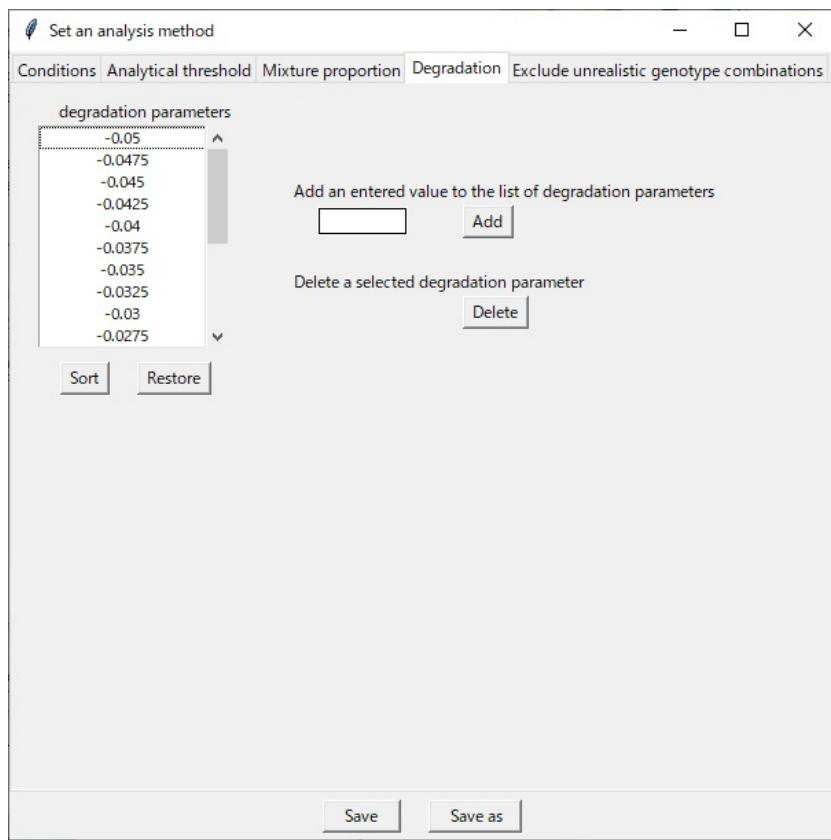
$$0 < MP_1 \leq MP_2 \leq \dots \leq MP_{N-1} \leq MP_N (N \geq 2).$$

In the "Mixture proportion" tab, candidate values for $MP_n (n = 1, 2, \dots, N - 1)$ can be set. MP_N is calculated as $1 - \sum_{n=1}^{N-1} MP_n$ in Kongoh.

Note

In addition to the sets of MP_n calculated by the above mentioned method, a set of the same amount of DNA in each contributor is automatically considered in Kongoh (e.g., $MP_1 = 0.33$, $MP_2 = 0.33$, $MP_3 = 0.34$ for $N = 3$).

"Degradation" tab

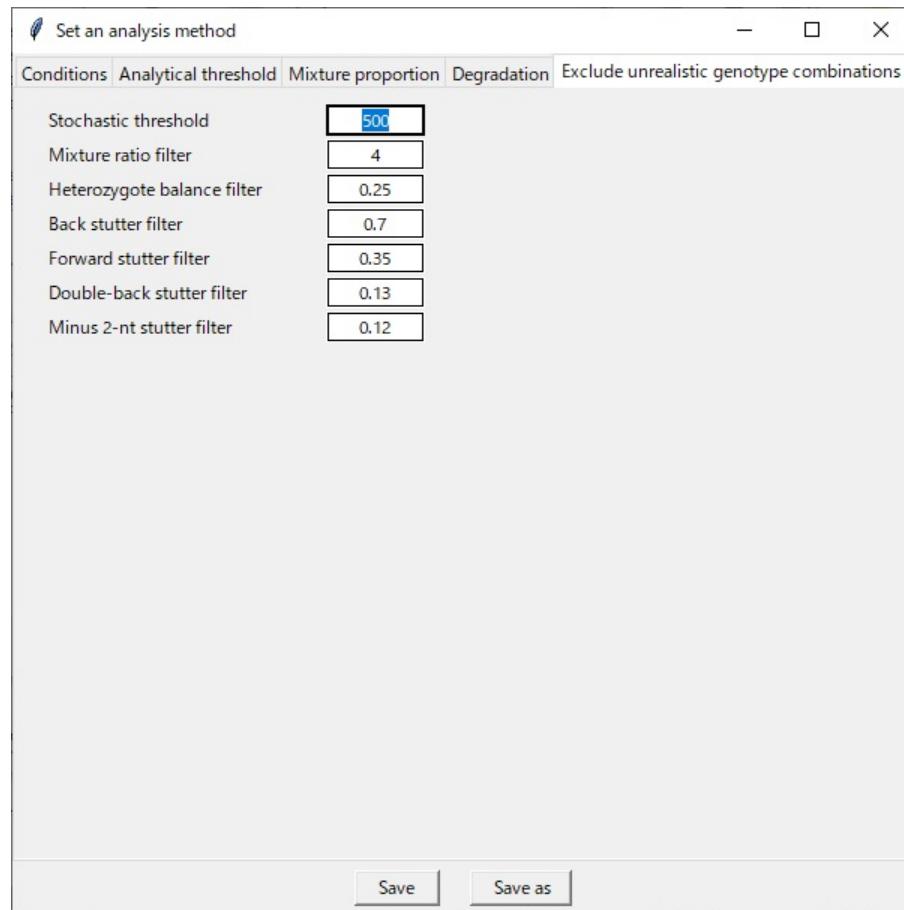


Users can manually add or delete the degradation parameters (d). $d = 0$ means no degradation. The smaller the d value, the greater the degree of degradation.

Note

The default range of d is $[-0.05, 0]$, which is discretized into 20 intervals of width 0.0025. When $d = -0.05$, DNA has significantly degraded. For example, when an allelic peak height located at the 100 base is 4,000 RFU, the peak height of the other allele located at the 225 base (which is approximately the middle detection point in the Identifiler Plus system) is expected to be only 8 RFU (i.e., typically less than the analytical threshold).

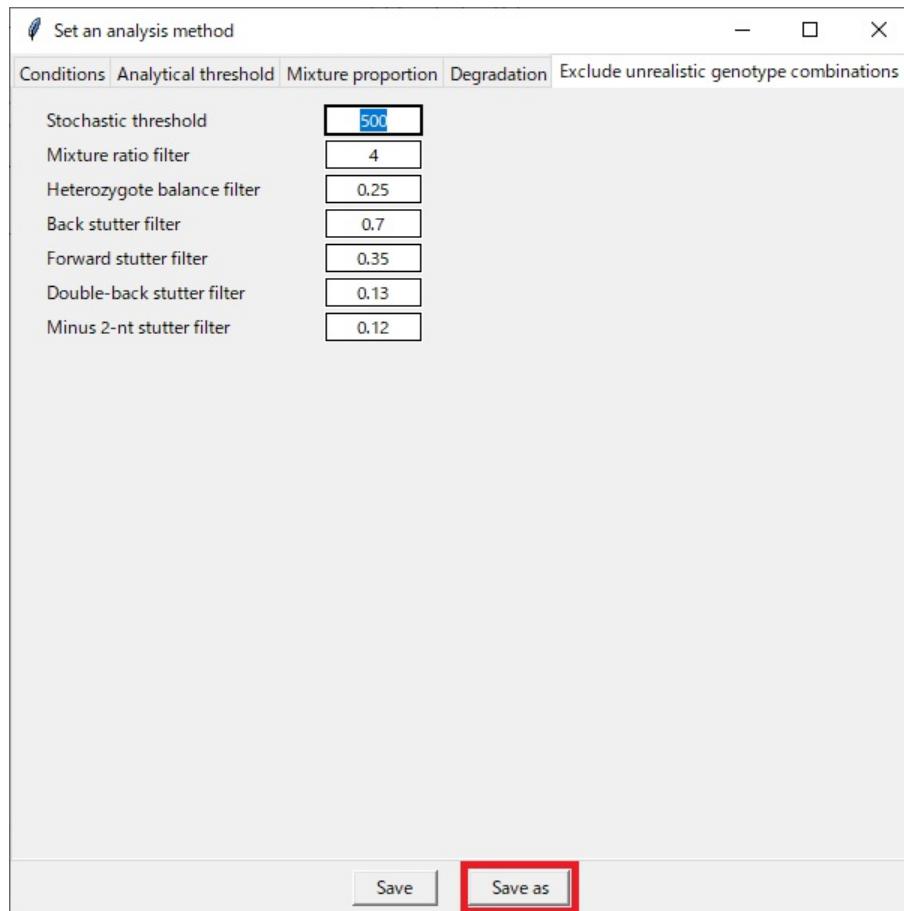
"Exclude unrealistic genotype combinations" tab



- **Stochastic threshold:** The mixture ratio filter and the heterozygote balance filter do not work if one of the peaks is below the threshold.
- **Mixture ratio filter:** A candidate genotype combination of minor and major contributors is inappropriate when the peak height of the minor (h_{minor}) is much larger than that of the major (h_{major}). If h_{minor}/h_{major} is greater than the mixture ratio filter, the genotype combination is excluded. h_{minor} and h_{major} are exactly the mean peak heights of the unique alleles in each.
- **Heterozygote balance filter:** If the peak height ratio of the two arbitrary peaks is smaller than the set value, the two peaks are not regarded as the heterozygote derived only from a single contributor.
- **Back stutter filter:** If the back stutter ratio is greater than the set value, the peak in the position of the back stutter is not regarded as the product derived only from the back stutter.
- **Forward stutter filter:** If the forward stutter ratio is greater than the set value, the peak in the position of the forward stutter is not regarded as the product derived only from the forward stutter.

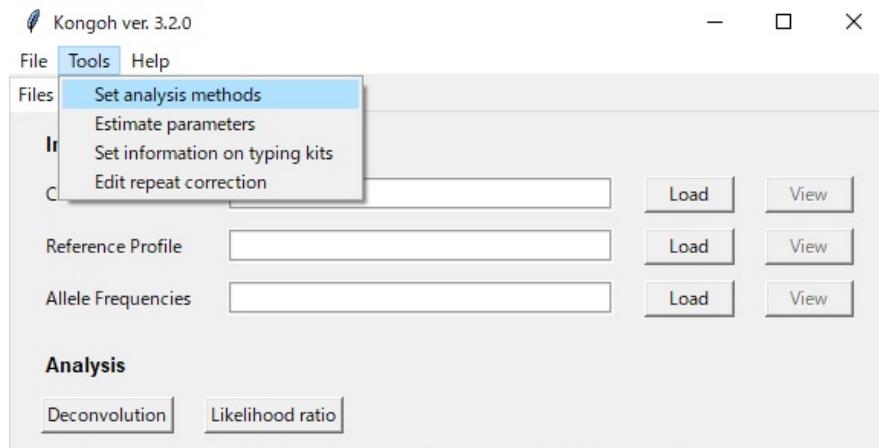
- **Double-back stutter filter:** If the double-back stutter ratio is greater than the set value, the peak in the position of the double-back stutter is not regarded as the product derived only from the double-back stutter.
- **Minus 2-nt stutter filter:** If the minus 2-nt stutter ratio is greater than the set value, the peak in the position of the minus 2-nt stutter is not regarded as the product derived only from the minus 2-nt stutter.

4. Click the "Save as" button after finishing all the settings.

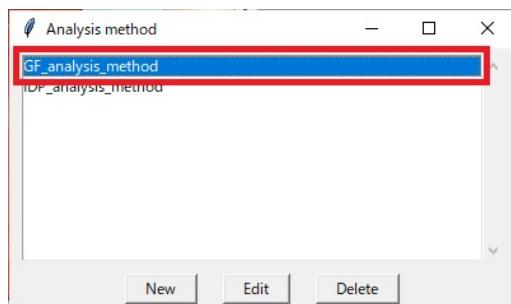


Edit an analysis method

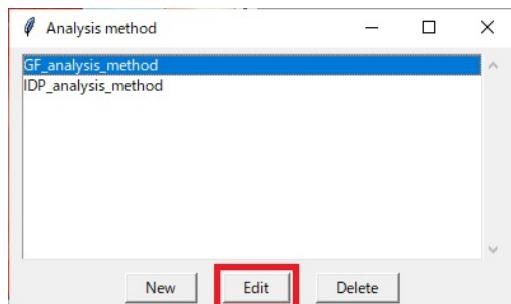
1. Go to Tools > Set analysis methods.



2. Select an analysis method.

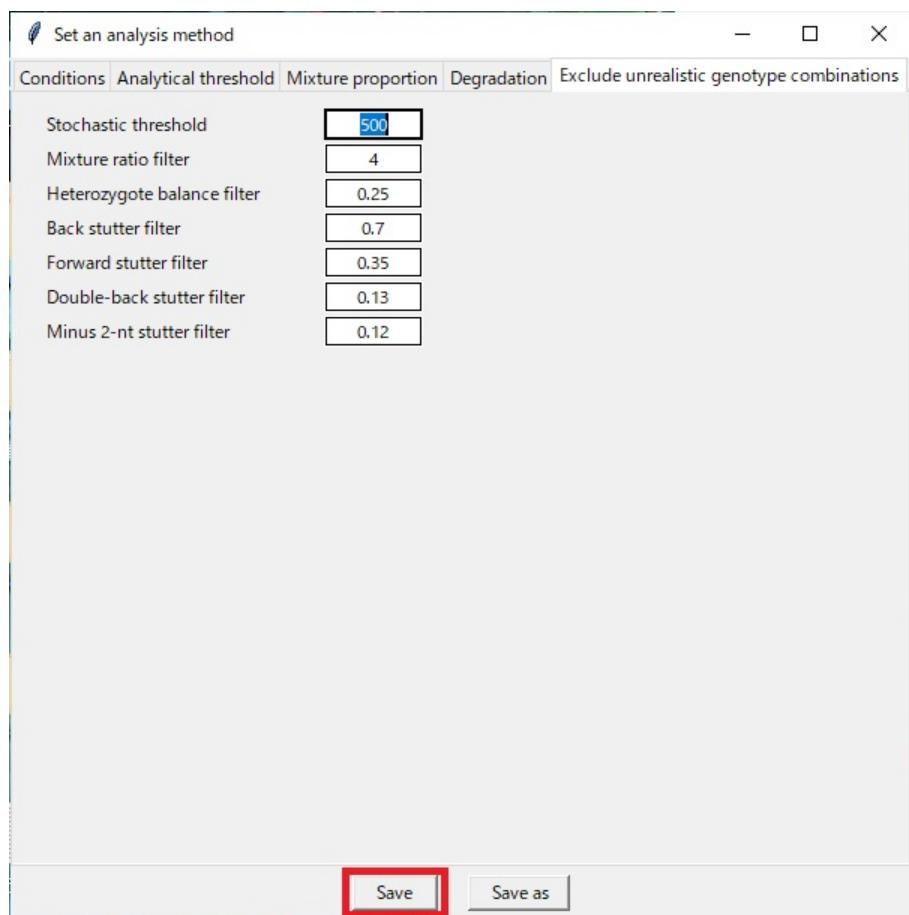


3. Click the "Edit" button to edit the selected analysis method.



4. Enter or select the settings just like creating an analysis method.

5. Click the "Save" button after finishing all the settings.

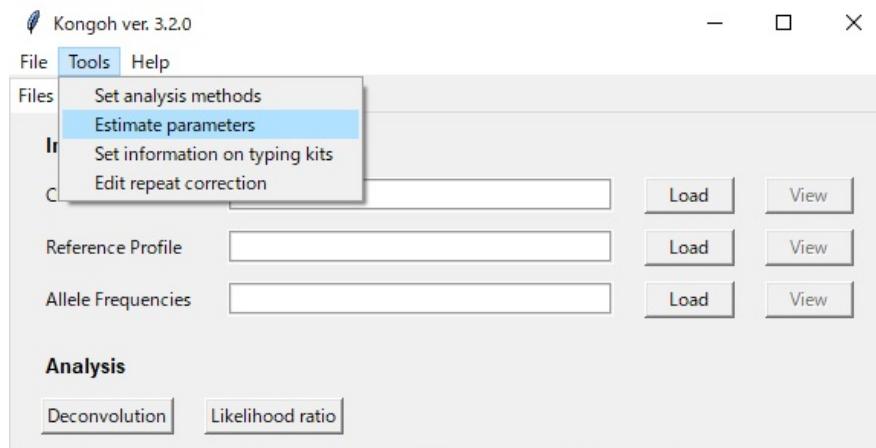


Estimate parameters

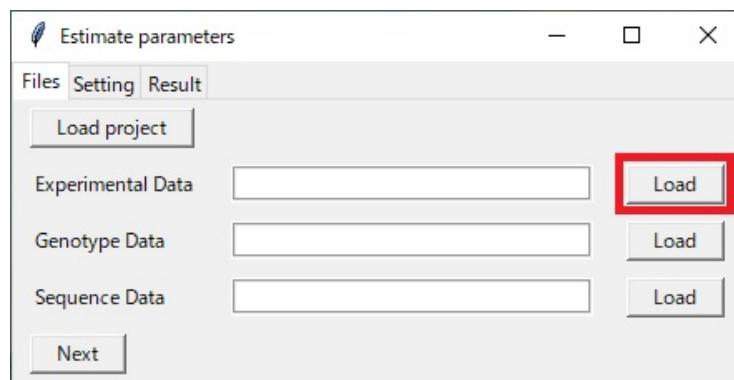
In Kongoh, probability distribution models for locus-specific amplification efficiency (AE), heterozygote balance (Hb), back stutter ratio (BSR), forward stutter ratio (FSR), double-back stutter ratio (DSR), and minus 2-nt stutter ratio (M2SR) can be estimated using experimental data prepared by users. The maximum likelihood estimation (MLE) for each parameter of the probability distribution models (e.g., mean, variance, etc.) is performed by the generalized simulated annealing, which is implemented into the R package “GenSA”. In this section, the procedure for the estimation of parameters is described.

Input files

1. Go to Tools > Estimate parameters.



2. Load a file of the experimental data.



Note

The experimental data should satisfy the following conditions:

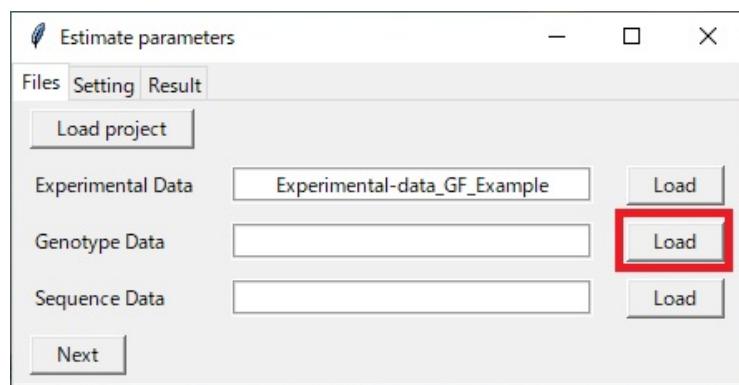
- The data are composed of single-source profiles.
- The profiles are derived from pristine (non-degraded) DNA samples.
- DNA samples should be prepared to the maximum extent, including samples of various amounts of DNA and various individuals.
- All DNA samples are analyzed based on one protocol.
- No filters are to be used to remove stutter peaks when analyzing sample electrophoretic data in software programs such as the GeneMapper® ID-X software.
- It is desirable to remove pull-up peaks and noises manually, but not required⁷.

Note

The example file "Experimental-data_GF_Example.csv" is provided. Go to extdata > example in the package *Kongoh*.

- The file of the experimental data can be exported from the GeneMapper® ID-X software.
- The file of the experimental data must include information regarding the "Sample Name", "Marker", "Allele", "Size", and "Height".

3. Load a file of the genotype data.



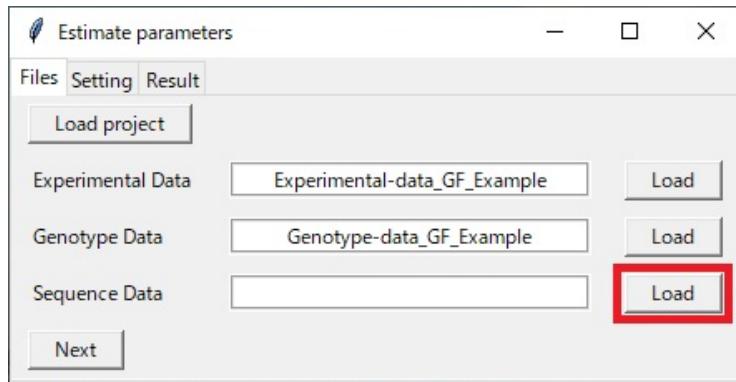
⁷Kongoh can automatically determine allele and stutter peaks based on the genotypes of each experimental data, as well as remove peaks located within ± 1 base[6] of an allele peak(s) in different color channels to exclude the effect of the spectral pull-up.

Note

The example file "Genotype-data_GF_Example.csv" is provided. Go to extdata > example in the package *Kongoh*.

- This file must include information regarding the "Sample Name", "Marker", "Allele 1", and "Allele 2".
- Information regarding the "Sample Name" in the "Genotype Data" file must be the same as that in the "Experimental Data" file.
- Two alleles in homozygotes must be entered in each column (except for Yindel and DYS391).

4. Load a file of the sequence data.



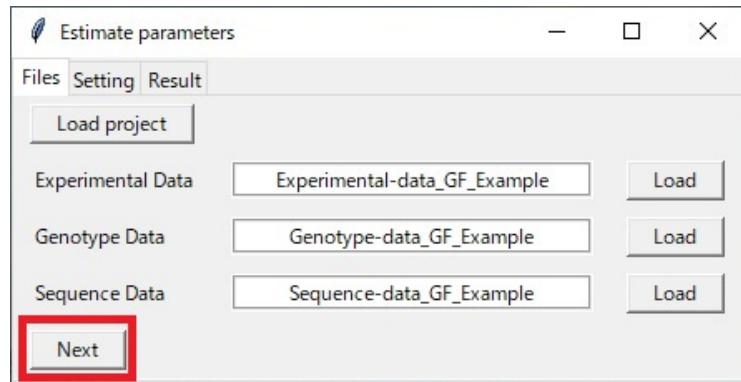
Note

The example file "Sequence-data_GF_Example.csv" is provided⁸. Go to extdata > example in the package *Kongoh*.

- This file must include information regarding the "Marker", "Allele", "Repeat Region", and "Count". Information on the "Flanking Region" is optional.
- "Count" implies the number of observations in sequence-based population data.

⁸The example data is derived from sequence-based U.S. population data for SE33[7] and for loci other than SE33[8].

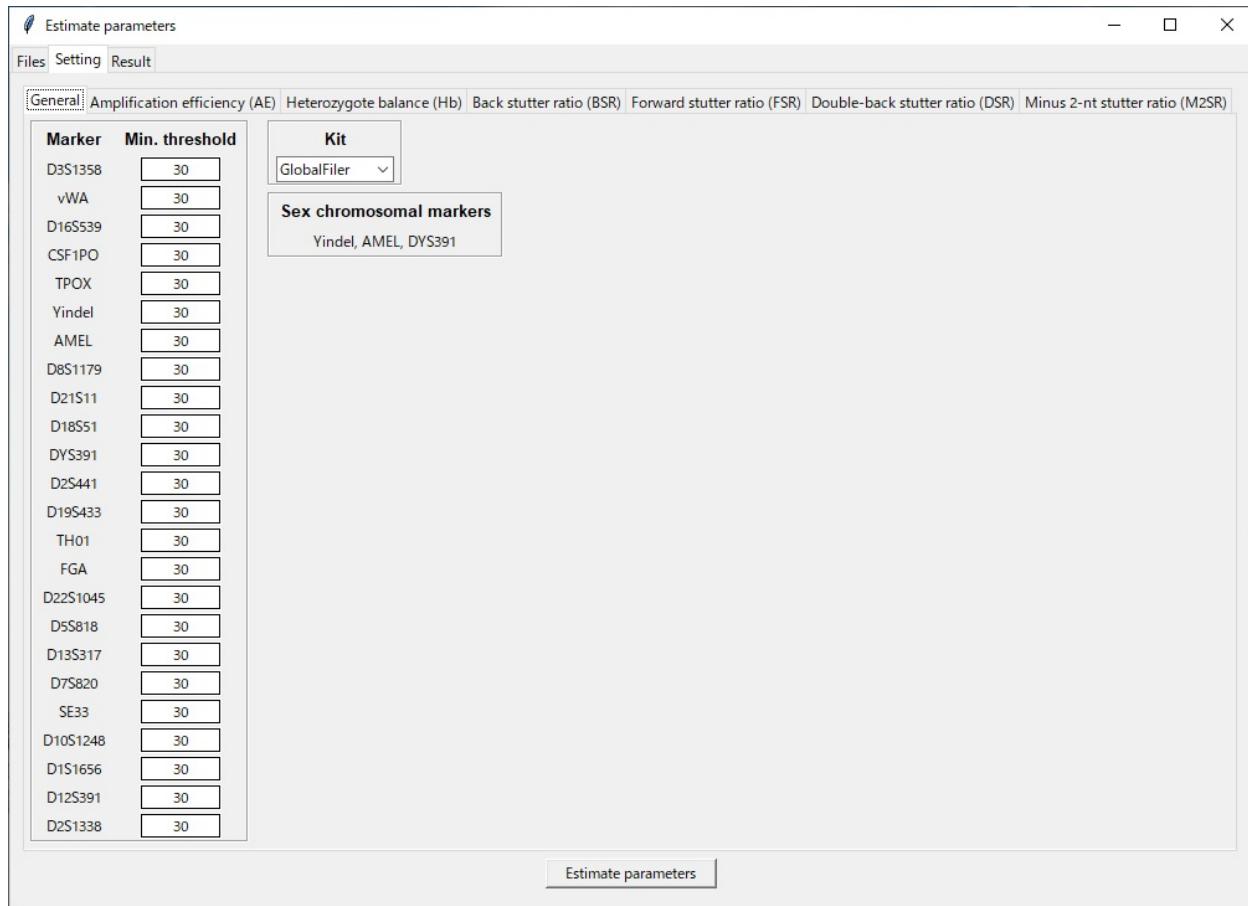
5. Click the "Next" button. Then the "Setting" tab will be automatically opened.



Set conditions

1. Set conditions shown in the figures on the following pages.

"General" tab



Min. threshold

Peaks below the thresholds are not used to estimate the parameters.

Kit

The kit name is automatically selected based on the input files. Users should select the appropriate kit name when more than one kit with the same locus set is registered in the feature "Edit information on typing kits"⁹ (e.g., Identifiler and Identifiler Plus).

⁹See the section "Edit information on typing kits" in the user manual.

”Amplification efficiency (AE)” tab

The screenshot shows the Kongoh software interface with the "Amplification efficiency (AE)" tab selected. The left panel lists markers with their respective models: D3S1358, vWA, D16S539, CSF1PO, TPOX, D8S1179, D21S11, D18S51, D2S441, D19S433, TH01, FGA, D22S1045, D5S818, D13S317, D7S820, SE33, D10S1248, D1S1656, D12S391, and D2S1338, all set to "Log-normal". In the center, under "Min. AE", there is a checked checkbox "Based on experimental data" with a value of 0.058. Below it, the "Conditions of MLE" section shows "Locus: D3S1358" and a table for parameters:

| Model | Parameter | Initial value | Lower value | Upper value |
|------------|-----------|---------------|-------------|-------------|
| Log-normal | Mean | 1 | -0.5 | 2 |
| | Variance | 50 | 1 | 500 |

At the bottom right is an "Estimate parameters" button.

Model

The model for AE is fixed to the “Log-normal” distribution in the current version of Kongoh.

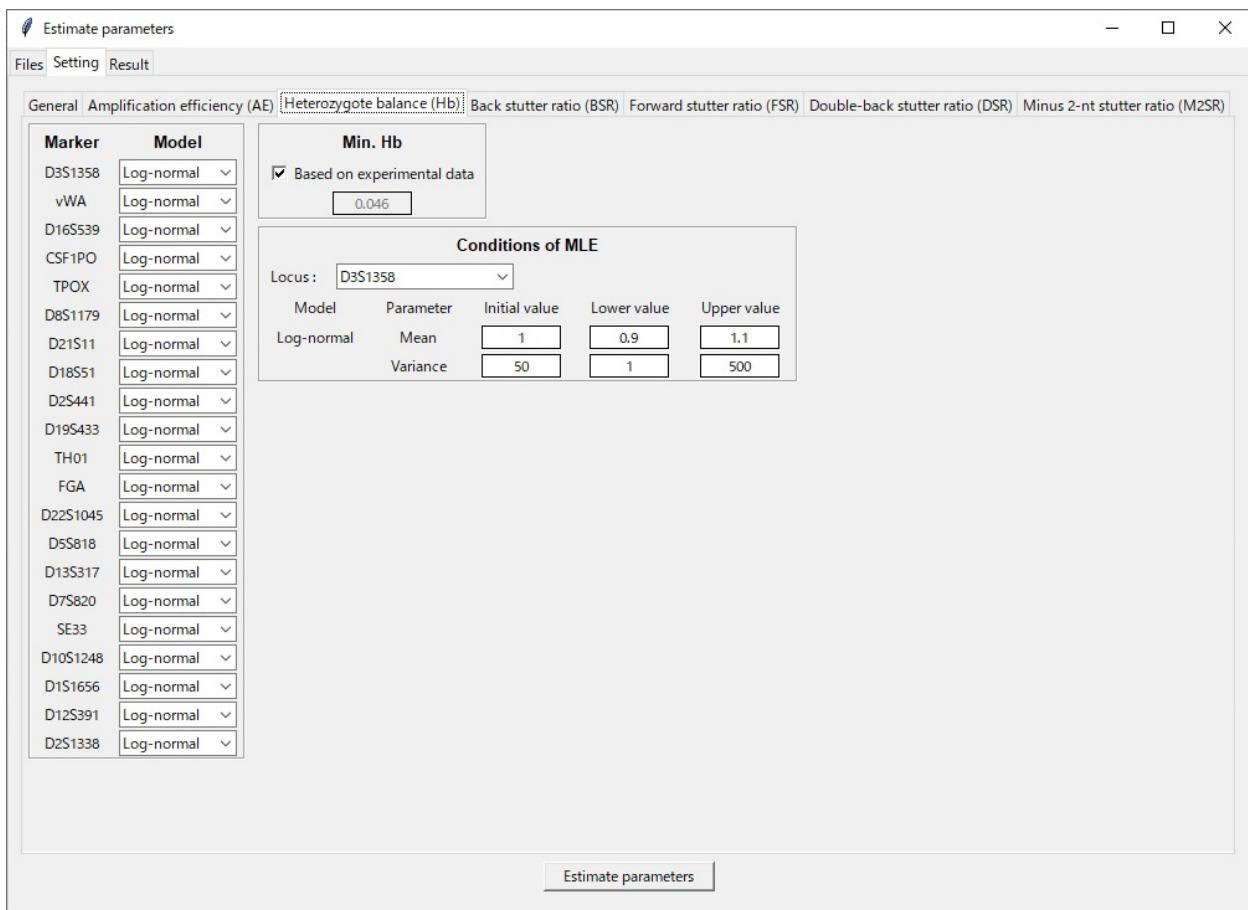
Min. AE

Min. AE is the lower limit of the truncated log-normal distribution for AE. The upper limit is automatically set to 1 / Min. AE. If ”Based on experimental data” is checked, Min. AE is set to the minimum of the experimental AE values. If ”Based on experimental data” is unchecked, users can enter an arbitrary value for Min. AE.

Conditions of MLE

Users can set initial, lower, and upper values of each parameter in the maximum likelihood estimation (MLE). These values do not need to be changed in the initial analysis of MLE. If the estimated probability distribution does not fit to the experimental data, users may try to change these values.

"Heterozygote balance (Hb)" tab



Model

The model for Hb is fixed to the “Log-normal” distribution in the current version of Kongoh.

Min. Hb

Min. Hb is the lower limit of the truncated log-normal distribution for Hb. The upper limit is automatically set to 1 / Min. Hb. If "Based on experimental data" is checked, Min. Hb is set to the minimum of the experimental Hb values. If "Based on experimental data" is unchecked, users can enter an arbitrary value for Min. Hb.

Conditions of MLE

Users can set initial, lower, and upper values of each parameter in the maximum likelihood estimation (MLE). These values do not need to be changed in the initial analysis of MLE. If the estimated probability distribution does not fit to the experimental data, users may try to change these values.

"Back stutter ratio (BSR)" tab

Model

There are four options for the model for BSR.

- **Best:** The best model is selected from Allele, LUS, and Multi-seq models based on the AIC (Akaike information criterion) values of each model.
- **Allele:** The BSRs are positively correlated with the allele numbers[9].
- **LUS:** The BSRs are positively correlated with the longest uninterrupted stretch (LUS) values[10, 11].
- **Multi-seq:** The BSRs are positively correlated with the corrected allele numbers, which consider not only the LUS, but also other uninterrupted stretches[12].

Method

There are three options for the methods of modeling.

- **Locus specific:** The experimental data of the locus is only used for estimating the probability distribution for this locus.

- **Multiple loci together:** The experimental data of all loci with the option "Multiple loci together" is used for estimating the probability distribution for these loci.
- **Not consider:** The probability distribution model is not considered in the locus.

Model when considering multiple loci together

Users can select models from four options (i.e., Best, Allele, LUS, and Multi-seq when considering multiple loci together).

Max. BSR

Max. BSR is the upper limit of the truncated log-normal distribution for BSR. If "Based on experimental data" is checked, Max. BSR is set to the maximum of the experimental BSRs in all loci. If "Based on experimental data" is unchecked, users can enter an arbitrary value for Max. BSR.

Conditions of MLE

Users can set initial, lower, and upper values of each parameter in the maximum likelihood estimation (MLE). These values do not need to be changed in the initial analysis of MLE. If the estimated probability distribution does not fit to the experimental data, users may try to change these values.

"Forward stutter ratio (FSR)" tab

| Marker | Model | Method |
|----------|------------------------|------------------------|
| D3S1358 | Multiple loci together | Multiple loci together |
| vWA | Multiple loci together | Multiple loci together |
| D16S539 | Multiple loci together | Multiple loci together |
| CSF1PO | Multiple loci together | Multiple loci together |
| TPOX | Multiple loci together | Multiple loci together |
| D8S1179 | Multiple loci together | Multiple loci together |
| D21S11 | Multiple loci together | Multiple loci together |
| D18S51 | Multiple loci together | Multiple loci together |
| D2S441 | Multiple loci together | Multiple loci together |
| D19S433 | Multiple loci together | Multiple loci together |
| TH01 | Multiple loci together | Multiple loci together |
| FGA | Multiple loci together | Multiple loci together |
| D22S1045 | Best | Locus specific |
| D5S818 | Multiple loci together | Multiple loci together |
| D13S317 | Multiple loci together | Multiple loci together |
| D7S820 | Multiple loci together | Multiple loci together |
| SE33 | Multiple loci together | Multiple loci together |
| D10S1248 | Multiple loci together | Multiple loci together |
| D1S1656 | Multiple loci together | Multiple loci together |
| D12S391 | Multiple loci together | Multiple loci together |
| D2S1338 | Multiple loci together | Multiple loci together |

Model

There are five options for the model for FSR.

- **Best:** The best model is selected from Allele, LUS, Multi-seq, and Uniform models based on the AIC values of each model.
- **Allele:** The FSRs are positively correlated with the allele numbers.
- **LUS:** The FSRs are positively correlated with the longest uninterrupted stretch (LUS) values.
- **Multi-seq:** The FSRs are positively correlated with the corrected allele numbers, which consider not only the LUS, but also other uninterrupted stretches.
- **Uniform:** The mean value of the FSRs is uniform regardless of the allele number.

Method

There are three options for the methods of modeling.

- **Locus specific:** The experimental data of the locus is only used for estimating the probability distribution for this locus.

- **Multiple loci together:** The experimental data of all loci with the option "Multiple loci together" is used for estimating the probability distribution for these loci.
- **Not consider:** The probability distribution model is not considered in the locus.

Model when considering multiple loci together

Users can select models from five options (i.e., Best, Allele, LUS, Multi-seq, and Uniform when considering multiple loci together).

Max. FSR

Max. FSR is the upper limit of the truncated log-normal distribution for FSR. If "Based on experimental data" is checked, Max. FSR is set to the maximum of the experimental FSRs in all loci. If "Based on experimental data" is unchecked, users can enter an arbitrary value for Max. FSR.

Conditions of MLE

Users can set initial, lower, and upper values of each parameter in the maximum likelihood estimation (MLE). These values do not need to be changed in the initial analysis of MLE. If the estimated probability distribution does not fit to the experimental data, users may try to change these values.

"Double-back stutter ratio (DSR)" tab

| Marker | Model | Method |
|----------|-------|------------------------|
| D3S1358 | ▼ | Multiple loci together |
| vWA | ▼ | Multiple loci together |
| D16S539 | ▼ | Multiple loci together |
| CSF1PO | ▼ | Multiple loci together |
| TPOX | ▼ | Multiple loci together |
| D8S1179 | ▼ | Multiple loci together |
| D21S11 | ▼ | Multiple loci together |
| D18S51 | ▼ | Multiple loci together |
| D2S441 | ▼ | Multiple loci together |
| D19S433 | ▼ | Multiple loci together |
| TH01 | ▼ | Multiple loci together |
| FGA | ▼ | Multiple loci together |
| D22S1045 | ▼ | Multiple loci together |
| D5S818 | ▼ | Multiple loci together |
| D13S317 | ▼ | Multiple loci together |
| D7S820 | ▼ | Multiple loci together |
| SE33 | ▼ | Multiple loci together |
| D10S1248 | ▼ | Multiple loci together |
| D1S1656 | ▼ | Multiple loci together |
| D12S391 | ▼ | Multiple loci together |
| D2S1338 | ▼ | Multiple loci together |

Model when considering multiple loci together
Best

Max. DSR
 Based on experimental data
0.13

Conditions of MLE
Locus: D3S1358

| Model | Parameter | Initial value | Lower value | Upper value |
|-----------|-----------|---------------|-------------|-------------|
| Allele | Slope | 0.005 | 0 | 0.1 |
| | Intercept | -0.01 | -1 | 1 |
| | Variance | 1000 | 1 | 5000 |
| LUS | Slope | 0.005 | 0 | 0.1 |
| | Intercept | -0.01 | -1 | 1 |
| | Variance | 1000 | 1 | 5000 |
| Multi-seq | Slope | 0.005 | 0 | 0.1 |
| | Intercept | -0.01 | -1 | 1 |
| | Variance | 1000 | 1 | 5000 |
| Uniform | X_value | 2 | 2 | 30 |
| | Mean | 0.01 | 0 | 1 |
| | Variance | 500 | 1 | 5000 |

Estimate parameters

Model

There are five options for the model for DSR.

- **Best:** The best model is selected from Allele, LUS, Multi-seq, and Uniform models based on the AIC values of each model.
- **Allele:** The DSRs are positively correlated with the allele numbers.
- **LUS:** The DSRs are positively correlated with the longest uninterrupted stretch (LUS) values.
- **Multi-seq:** The DSRs are positively correlated with the corrected allele numbers, which consider not only the LUS, but also other uninterrupted stretches.
- **Uniform:** The mean value of the DSRs is uniform regardless of the allele number.

Method

There are three options for the methods of modeling.

- **Locus specific:** The experimental data of the locus is only used for estimating the probability distribution for this locus.

- **Multiple loci together:** The experimental data of all loci with the option "Multiple loci together" is used for estimating the probability distribution for these loci.
- **Not consider:** The probability distribution model is not considered in the locus.

Model when considering multiple loci together

Users can select models from five options (i.e., Best, Allele, LUS, Multi-seq, and Uniform when considering multiple loci together).

Max. DSR

Max. DSR is the upper limit of the truncated log-normal distribution for DSR. If "Based on experimental data" is checked, Max. DSR is set to the maximum of the experimental DSRs in all loci. If "Based on experimental data" is unchecked, users can enter an arbitrary value for Max. DSR.

Conditions of MLE

Users can set initial, lower, and upper values of each parameter in the maximum likelihood estimation (MLE). These values do not need to be changed in the initial analysis of MLE. If the estimated probability distribution does not fit to the experimental data, users may try to change these values.

"Minus 2-nt stutter ratio (M2SR)" tab

Model

The model for M2SR is fixed to the "Uniform" distribution in the current version of Kongoh.

Method

There are three options for the methods of modeling.

- **Locus specific:** The experimental data of the locus is only used for estimating the probability distribution for this locus.
- **Multiple loci together:** The experimental data of all loci with the option "Multiple loci together" is used for estimating the probability distribution for these loci.
- **Not consider:** The probability distribution model is not considered in the locus.

Model when considering multiple loci together

The model for M2SR is fixed to the "Uniform" distribution in the current version of Kongoh when considering multiple loci together.

Max. M2SR

Max. M2SR is the upper limit of the truncated log-normal distribution for M2SR. If "Based

on experimental data” is checked, Max. M2SR is set to the maximum of the experimental M2SRs in all loci. If ”Based on experimental data” is unchecked, users can enter an arbitrary value for Max. M2SR.

Conditions of MLE

Users can set initial, lower, and upper values of each parameter in the maximum likelihood estimation (MLE). These values do not need to be changed in the initial analysis of MLE. If the estimated probability distribution does not fit to the experimental data, users may try to change these values.

2. Click the ”Estimate parameters” button after setting all conditions.

The screenshot shows the 'Estimate parameters' software window. At the top, there are tabs for 'Files', 'Setting', and 'Result'. Below the tabs is a horizontal bar with several options: 'General', 'Amplification efficiency (AE)', 'Heterozygote balance (Hb)', 'Back stutter ratio (BSR)', 'Forward stutter ratio (FSR)', 'Double-back stutter ratio (DSR)', and 'Minus 2-nt stutter ratio (M2SR)'. The 'General' tab is selected. The main area contains a table with columns 'Marker' and 'Min. threshold'. The 'Marker' column lists various STR markers, and the 'Min. threshold' column shows '30' for all markers. To the right of the table is a 'Kit' section with a dropdown menu set to 'GlobalFiler'. Below the table is a box labeled 'Sex chromosomal markers' containing 'Yindel, AMEL, DYS391'. At the bottom right of the main area is a red-bordered 'Estimate parameters' button.

| Marker | Min. threshold |
|----------|----------------|
| D3S1358 | 30 |
| vWA | 30 |
| D16S539 | 30 |
| CSF1PO | 30 |
| TPOX | 30 |
| Yindel | 30 |
| AMEL | 30 |
| D8S1179 | 30 |
| D21S11 | 30 |
| D18S51 | 30 |
| DYS391 | 30 |
| D2S441 | 30 |
| D19S433 | 30 |
| TH01 | 30 |
| FGA | 30 |
| D22S1045 | 30 |
| D5S818 | 30 |
| D13S317 | 30 |
| D7S820 | 30 |
| SE33 | 30 |
| D10S1248 | 30 |
| D1S1656 | 30 |
| D12S391 | 30 |
| D2S1338 | 30 |

Note

It takes a few hours to estimate parameters. After the parameter estimation is completed, the “Result” tab appears automatically.

Review the results

1. Review the results of estimated parameters.

- Adopted models and estimated parameters in each locus are displayed on the left side of the screen.

The screenshot shows a software window titled "Estimate parameters". At the top, there are tabs for "Files", "Setting", and "Result". Below the tabs is a horizontal menu bar with buttons for "Amplification efficiency (AE)", "Heterozygote balance (Hb)", "Back stutter ratio (BSR)", "Forward stutter ratio (FSR)", "Double-back stutter ratio (DSR)", and "Minus 2-nt stutter ratio (M2SR)". The main area contains a table with data. A red box highlights the first few rows of the table. The table has columns: Marker, Adopted model, User-selected, Method, Max. BSR, Slope, Intercept, Variance, and X_value. The "Marker" column lists various STR loci. The "Adopted model" column includes "Multi-seq", "Allele", and "Locus specific". The "Method" column consistently shows "Locus specific". The "Max. BSR" column contains values like 0.192, 0.192, 0.192, etc. The "Slope" column contains values like 0.1, 0.0109, 0.0114, etc. The "Intercept" column contains values like -0.12, -0.0785, -0.0411, etc. The "Variance" column contains values like 687, 415, 37.1, etc. The "X_value" column contains values like 14, 12, 407, etc. At the bottom of the table area are three buttons: "Show detail", "Export", and "Save project".

| Marker | Adopted model | User-selected | Method | Max. BSR | Slope | Intercept | Variance | X_value |
|----------|---------------|---------------|----------------|----------|---------|-----------|----------|---------|
| D3S1358 | Multi-seq | No | Locus specific | 0.192 | 0.1 | 0.0451 | 687 | 14 |
| VWA | Allele | No | Locus specific | 0.192 | 0.0109 | -0.12 | 25.1 | |
| D16S539 | Allele | No | Locus specific | 0.192 | 0.0114 | -0.0785 | 415 | |
| CSF1PO | Allele | No | Locus specific | 0.192 | 0.00855 | -0.0411 | 37.1 | |
| TPOX | LUS | No | Locus specific | 0.192 | 0.00573 | -0.0294 | 44.9 | |
| D8S1179 | Multi-seq | No | Locus specific | 0.192 | 0.0125 | 0.0503 | 674 | 12 |
| D21S11 | LUS | No | Locus specific | 0.192 | 0.00636 | -0.0122 | 407 | |
| D18S51 | Allele | No | Locus specific | 0.192 | 0.00657 | -0.032 | 47.3 | |
| D2S441 | LUS | No | Locus specific | 0.192 | 0.00589 | -0.0235 | 357 | |
| D19S433 | Allele | No | Locus specific | 0.192 | 0.00696 | -0.0345 | 25.2 | |
| TH01 | Allele | No | Locus specific | 0.192 | 0.00269 | -0.0032 | 434 | |
| FGA | Allele | No | Locus specific | 0.192 | 0.00697 | -0.0871 | 18.5 | |
| D2S1045 | Allele | No | Locus specific | 0.192 | 0.00793 | -0.0673 | 596 | |
| D5S818 | LUS | No | Locus specific | 0.192 | 0.00914 | -0.0474 | 27.3 | |
| D13S317 | LUS | No | Locus specific | 0.192 | 0.0102 | -0.0623 | 104 | |
| D7S820 | Multi-seq | No | Locus specific | 0.192 | 0.00538 | 0.0239 | 422 | 9 |
| SE33 | Multi-seq | No | Locus specific | 0.192 | 0.00493 | 0.0445 | 133 | 9 |
| D10S1248 | Multi-seq | No | Locus specific | 0.192 | 0.0343 | 0.0415 | 592 | 15 |
| D1S1656 | Multi-seq | No | Locus specific | 0.192 | 0.037 | 0.061 | 268 | 15 |
| D12S391 | Multi-seq | No | Locus specific | 0.192 | 0.0393 | 0.0476 | 415 | 12 |
| D2S1338 | Allele | No | Locus specific | 0.192 | 0.00472 | -0.033 | 348 | |

- Detailed results of one locus can be displayed by selecting a locus and clicking the "Show detail" button.

Estimate parameters

Files Setting Result

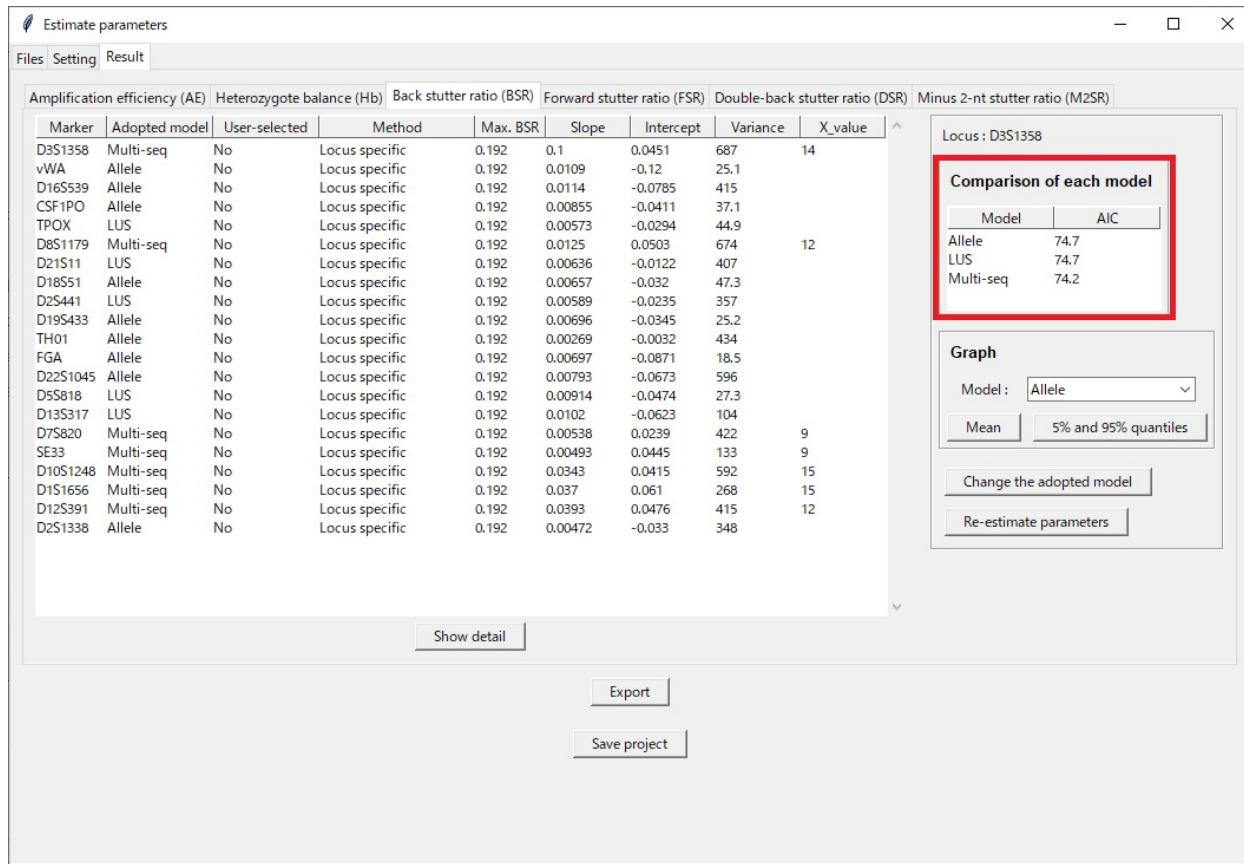
| Marker | | Adopted model | User-selected | Method | Max_BSR | Slope | Intercept | Variance | X value |
|----------|-----------|---------------|----------------|--------|---------|---------|-----------|----------|---------|
| D3S1358 | Multi-seq | No | Locus specific | 0.192 | 0.1 | 0.0451 | 687 | 14 | |
| VWA | Allele | No | Locus specific | 0.192 | 0.0109 | -0.12 | 25.1 | | |
| D16S539 | Allele | No | Locus specific | 0.192 | 0.0114 | -0.0785 | 415 | | |
| CSF1PO | Allele | No | Locus specific | 0.192 | 0.00855 | -0.0411 | 37.1 | | |
| TPOX | LUS | No | Locus specific | 0.192 | 0.00573 | -0.0294 | 44.9 | | |
| D8S1179 | Multi-seq | No | Locus specific | 0.192 | 0.0125 | 0.0503 | 674 | 12 | |
| D2S111 | LUS | No | Locus specific | 0.192 | 0.00636 | -0.0122 | 407 | | |
| D18S51 | Allele | No | Locus specific | 0.192 | 0.00657 | -0.032 | 47.3 | | |
| D2S441 | LUS | No | Locus specific | 0.192 | 0.00589 | -0.0235 | 357 | | |
| D19S433 | Allele | No | Locus specific | 0.192 | 0.00696 | -0.0345 | 25.2 | | |
| TH01 | Allele | No | Locus specific | 0.192 | 0.00269 | -0.0032 | 434 | | |
| FGA | Allele | No | Locus specific | 0.192 | 0.00697 | -0.0871 | 18.5 | | |
| D2S1045 | Allele | No | Locus specific | 0.192 | 0.00793 | -0.0673 | 596 | | |
| D5S818 | LUS | No | Locus specific | 0.192 | 0.00914 | -0.0474 | 27.3 | | |
| D13S317 | LUS | No | Locus specific | 0.192 | 0.0102 | -0.0623 | 104 | | |
| D7S820 | Multi-seq | No | Locus specific | 0.192 | 0.00538 | 0.0239 | 422 | 9 | |
| SE33 | Multi-seq | No | Locus specific | 0.192 | 0.00493 | 0.0445 | 133 | 9 | |
| D10S1248 | Multi-seq | No | Locus specific | 0.192 | 0.0343 | 0.0415 | 592 | 15 | |
| D1S1656 | Multi-seq | No | Locus specific | 0.192 | 0.037 | 0.061 | 268 | 15 | |
| D12S391 | Multi-seq | No | Locus specific | 0.192 | 0.0393 | 0.0476 | 415 | 12 | |
| D2S1338 | Allele | No | Locus specific | 0.192 | 0.00472 | -0.033 | 348 | | |

Show detail

Export

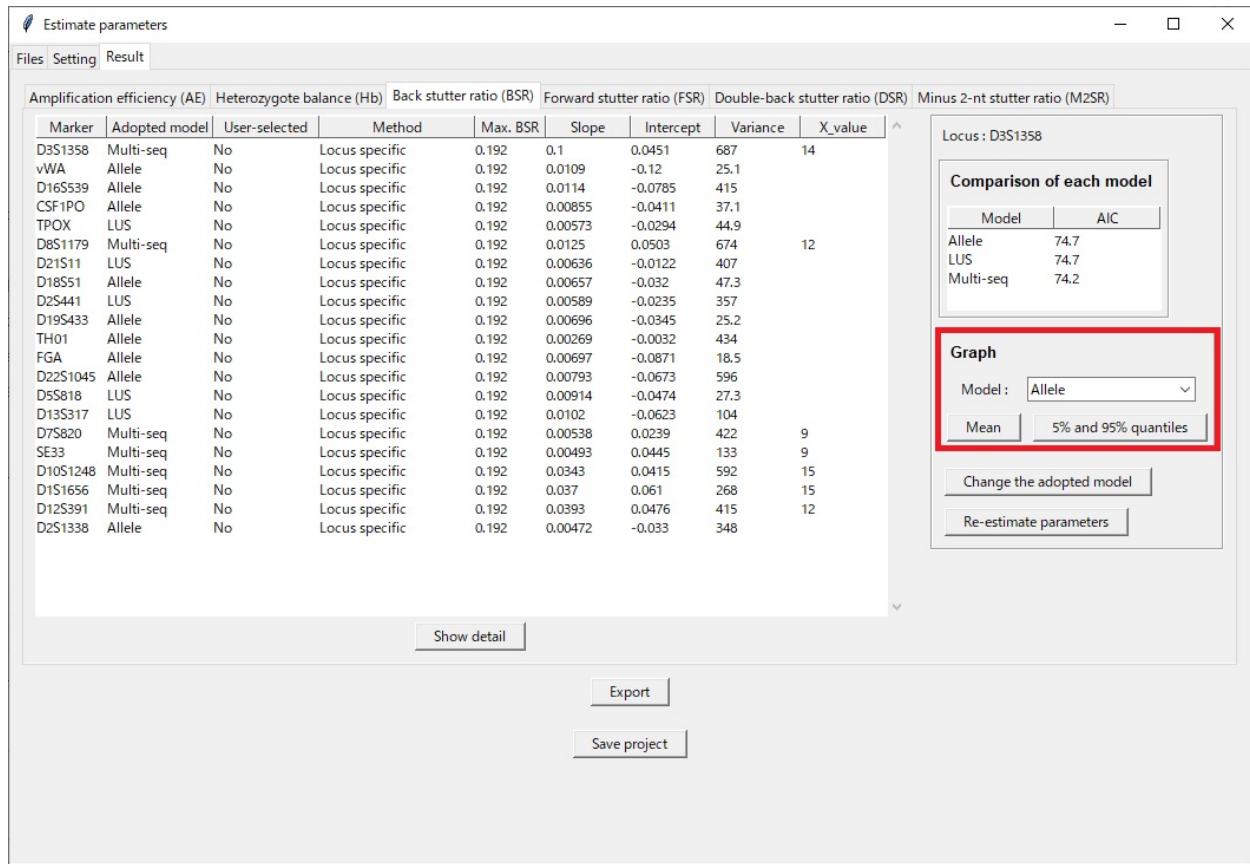
Save project

- AIC (Akaike information criterion) values¹⁰ of each model are displayed in the frame of "Comparison of each model".



¹⁰A model featuring a low AIC is considered a superior fit for the experimental SR data as compared with a model featuring a high AIC.

- Users can verify whether the estimated parameters are fitted to the experimental data. The graph for "5% and 95% quantiles" can be described in terms of the AE and Hb. The graphs for "Mean" and "5% and 95% quantiles" can be described in terms of the BSR, FSR, DSR, and M2SR. For example, the graphs for "Mean" and "5% and 95% quantiles" of BSR can be described as shown in Figures 6 and 7, respectively.



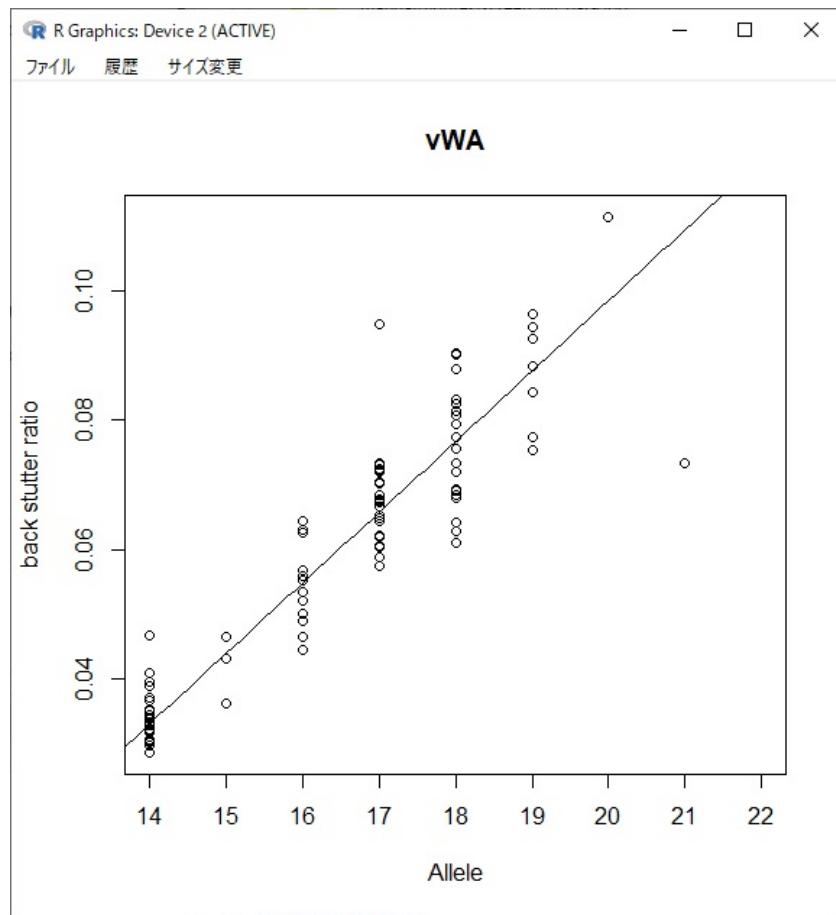


Figure 7: An example of the graph for "Mean" of back stutter ratios. The solid line indicates a regression line of mean values.

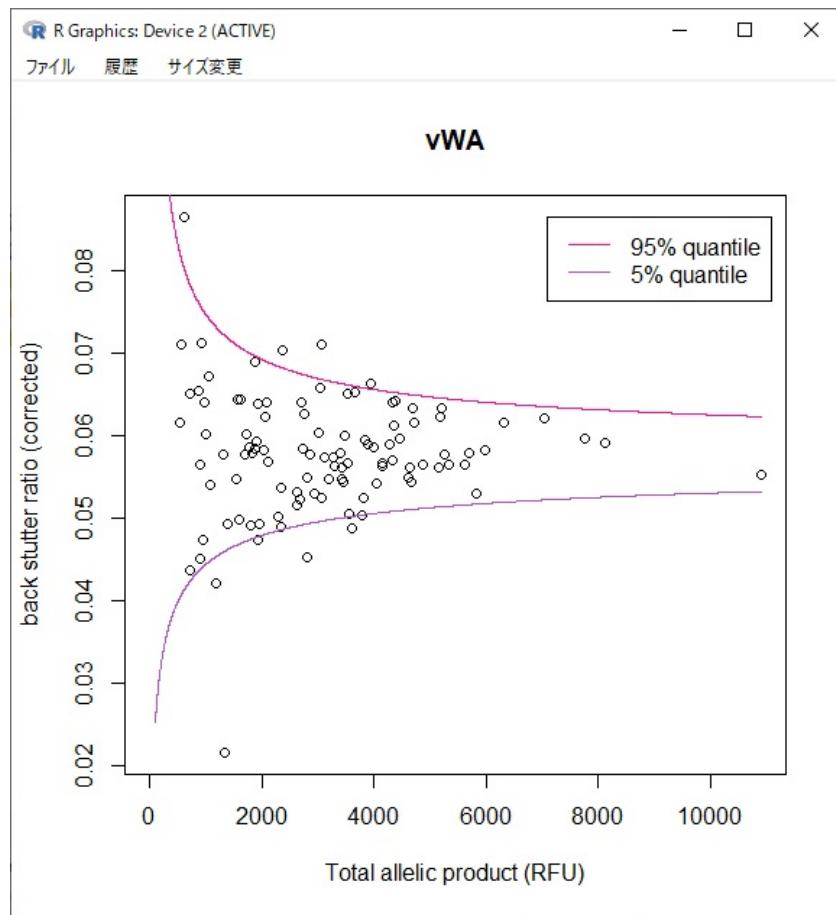
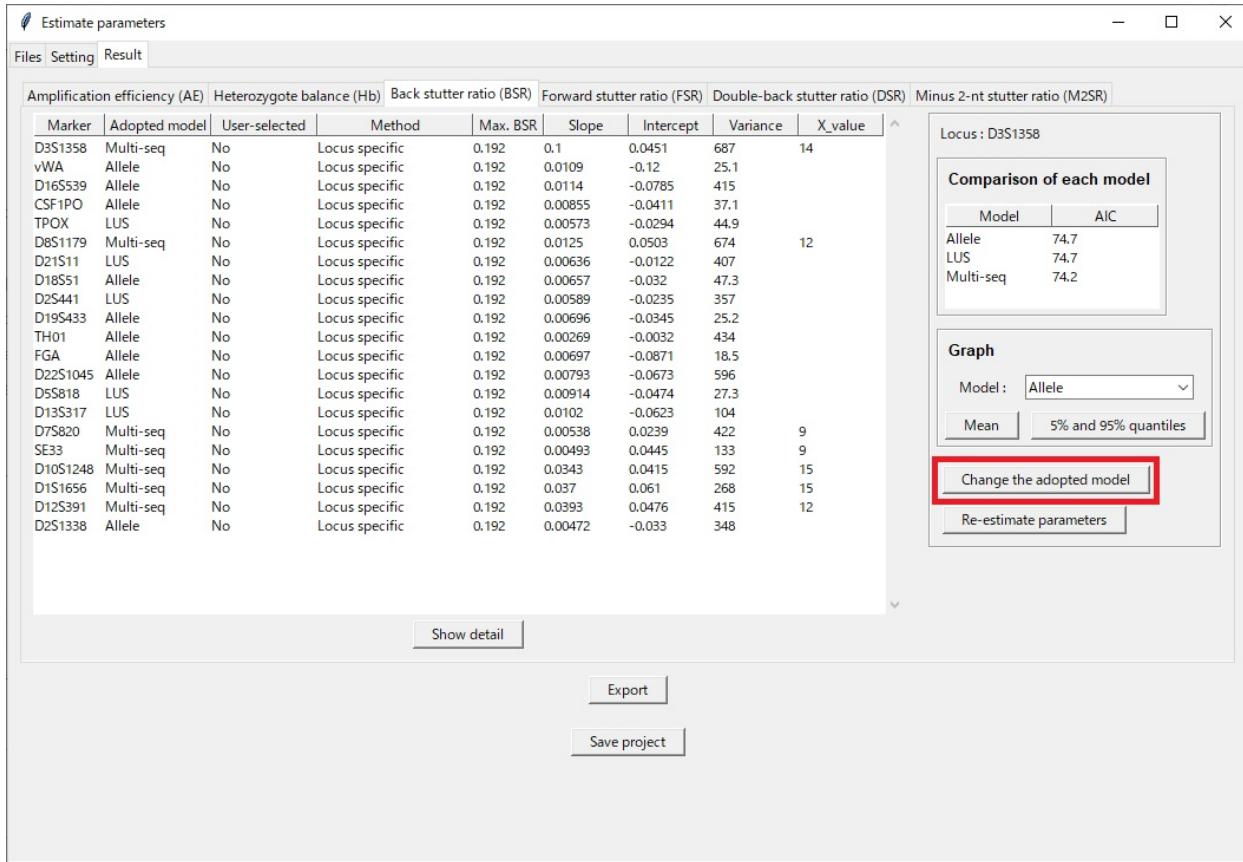
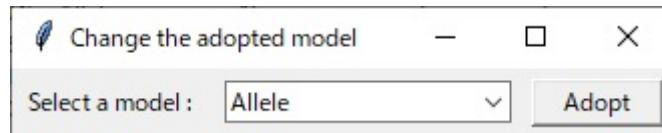


Figure 8: An example of the graph for "5% and 95% quantiles" of back stutter ratios. The relationship between back stutter ratios and total allelic products is shown. The upper and lower solid lines indicate 95% and 5% quantiles of back stutter ratios, respectively. Back stutter ratios are corrected by eliminating the difference in mean values in each allele.

- Users can change the adopted model for BSR, FSR, and DSR by clicking the "Change the adopted model" button.



Then, a new window will appear. Select the desired model, and click the “Adopt” button.



The user-selected model will be reflected and the "User-selected" column of the target locus is changed to "Yes".

Estimate parameters

Files Setting Result

Amplification efficiency (AE) Heterozygote balance (Hb) Back stutter ratio (BSR) Forward stutter ratio (FSR) Double-back stutter ratio (DSR) Minus 2-nt stutter ratio (M2SR)

| Marker | Adopted model | User-selected | Method | Max. BSR | Slope | Intercept | Variance | X_value |
|----------|---------------|---------------|----------------|----------|---------|-----------|----------|---------|
| D3S1358 | LUS | Yes | Locus specific | 0.192 | 0 | 0.046 | 716 | |
| vWA | Allele | No | Locus specific | 0.192 | 0.0109 | -0.12 | 25.1 | |
| D16S539 | Allele | No | Locus specific | 0.192 | 0.0114 | -0.0785 | 415 | |
| CSF1PO | Allele | No | Locus specific | 0.192 | 0.00855 | -0.0411 | 37.1 | |
| TPOX | LUS | No | Locus specific | 0.192 | 0.00573 | -0.0294 | 44.9 | |
| D8S1179 | Multi-seq | No | Locus specific | 0.192 | 0.0125 | 0.0503 | 674 | 12 |
| D2S111 | LUS | No | Locus specific | 0.192 | 0.00636 | -0.0122 | 407 | |
| D18S51 | Allele | No | Locus specific | 0.192 | 0.00657 | -0.032 | 47.3 | |
| D2S441 | LUS | No | Locus specific | 0.192 | 0.00589 | -0.0235 | 357 | |
| D19S433 | Allele | No | Locus specific | 0.192 | 0.00696 | -0.0345 | 25.2 | |
| TH01 | Allele | No | Locus specific | 0.192 | 0.00269 | -0.0032 | 434 | |
| FGA | Allele | No | Locus specific | 0.192 | 0.00697 | -0.0871 | 18.5 | |
| D22S1045 | Allele | No | Locus specific | 0.192 | 0.00793 | -0.0673 | 596 | |
| D5S818 | LUS | No | Locus specific | 0.192 | 0.00914 | -0.0474 | 27.3 | |
| D13S317 | LUS | No | Locus specific | 0.192 | 0.0102 | -0.0623 | 104 | |
| D7S820 | Multi-seq | No | Locus specific | 0.192 | 0.00538 | 0.0239 | 422 | 9 |
| SE33 | Multi-seq | No | Locus specific | 0.192 | 0.00493 | 0.0445 | 133 | 9 |
| D10S1248 | Multi-seq | No | Locus specific | 0.192 | 0.0343 | 0.0415 | 592 | 15 |
| D1S1656 | Multi-seq | No | Locus specific | 0.192 | 0.037 | 0.061 | 268 | 15 |
| D12S391 | Multi-seq | No | Locus specific | 0.192 | 0.0393 | 0.0476 | 415 | 12 |
| D2S1338 | Allele | No | Locus specific | 0.192 | 0.00472 | -0.033 | 348 | |

Locus : D3S1358

Comparison of each model

| Model | AIC |
|-----------|------|
| Allele | 74.7 |
| LUS | 74.7 |
| Multi-seq | 74.2 |

Graph

Model : Allele

Mean 5% and 95% quantiles

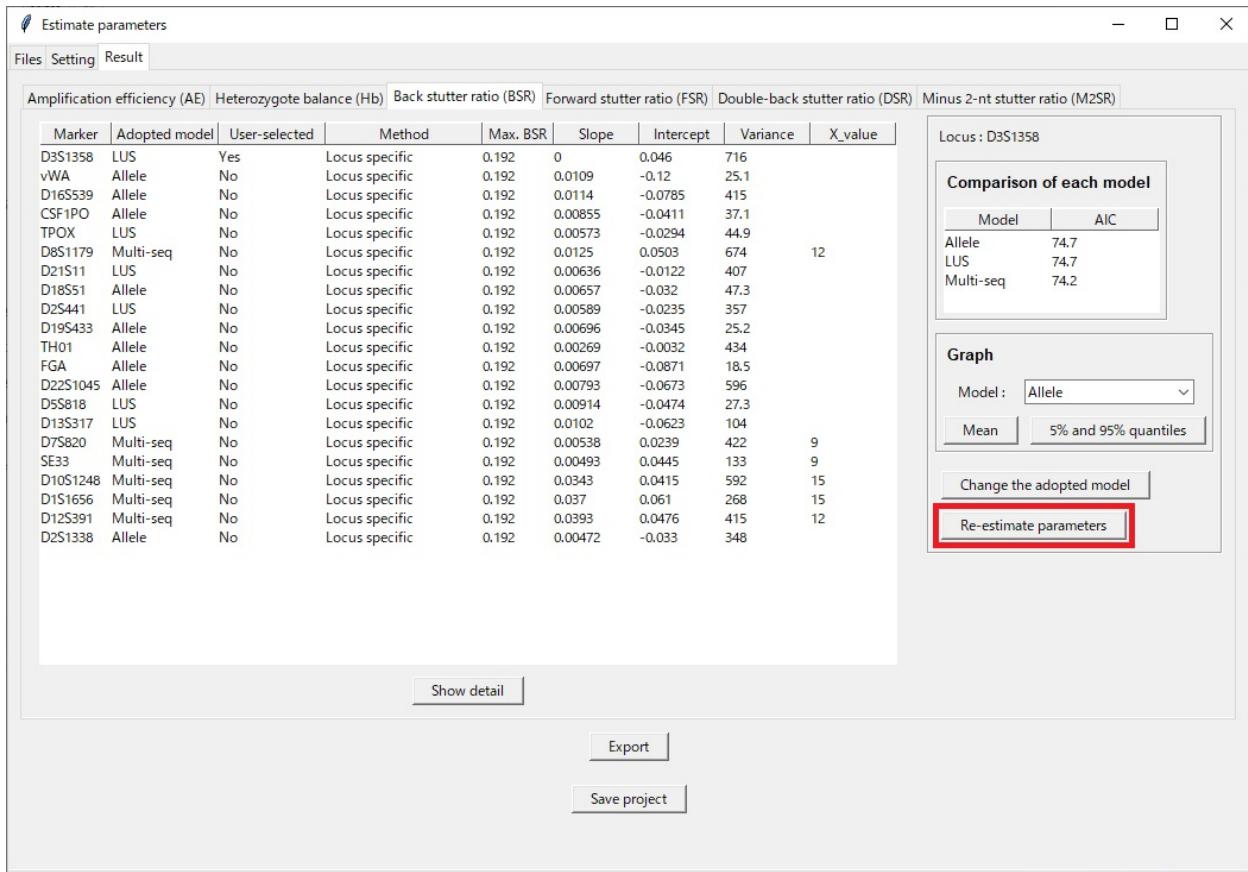
Change the adopted model Re-estimate parameters

Show detail

Export

Save project

- Users can re-estimate the parameters by clicking the "Re-estimate parameters" button.



Then, a new window will appear. Select a model and set initial, lower, and upper values of each parameter in the maximum likelihood estimation (MLE). After that, click the "Estimate" button to start the re-estimation.

The screenshot shows the 'Re-estimate parameters' dialog box. It has a dropdown menu 'Select a model : Allele'. Below it is a table with columns: Parameter, Initial value, Lower value, and Upper value. The parameters listed are Slope, Intercept, and Variance. The 'Estimate' button is at the bottom.

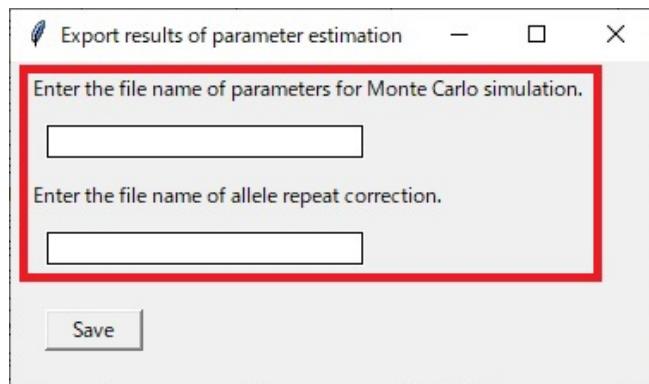
| Parameter | Initial value | Lower value | Upper value |
|-----------|---------------|-------------|-------------|
| Slope | 0.01 | 0 | 0.1 |
| Intercept | -0.05 | -1 | 1 |
| Variance | 50 | 1 | 1000 |

2. After reviewing all the results of estimated parameters, click the "Export" button.

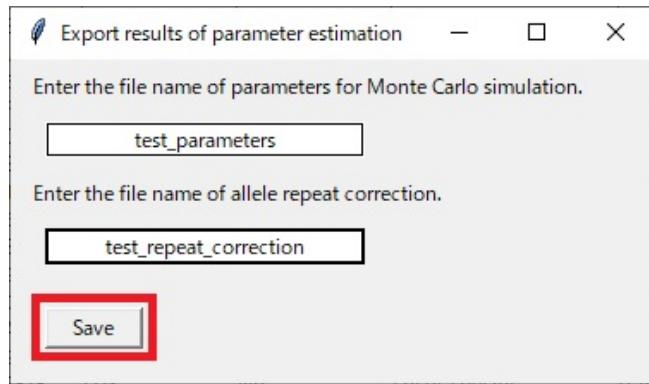
The screenshot shows the 'Estimate parameters' software window. At the top, there are tabs: 'Estimate parameters' (selected), 'Files', 'Setting', and 'Result'. Below the tabs is a horizontal bar with buttons: 'Amplification efficiency (AE)', 'Heterozygote balance (Hb)', 'Back stutter ratio (BSR)', 'Forward stutter ratio (FSR)' (selected), 'Double-back stutter ratio (DSR)', and 'Minus 2-nt stutter ratio (M2SR)'. The main area contains a table with the following columns: Marker, Adopted model, User-selected, Method, Max. BSR, Slope, Intercept, Variance, and X_value. The table lists various STR markers with their respective parameters. To the right of the table is a panel titled 'Locus : D3S1358' containing a 'Comparison of each model' table and a 'Graph' section. The 'Comparison of each model' table has columns 'Model' and 'AIC', listing 'Allele' (74.7), 'LUS' (74.7), and 'Multi-seq' (74.2). The 'Graph' section includes a dropdown 'Model:' set to 'Allele', and buttons for 'Mean' and '5% and 95% quantiles'. At the bottom of the main area are buttons for 'Show detail', 'Export' (highlighted with a red box), and 'Save project'.

| Marker | Adopted model | User-selected | Method | Max. BSR | Slope | Intercept | Variance | X_value |
|----------|---------------|---------------|----------------|----------|---------|-----------|----------|---------|
| D3S1358 | LUS | Yes | Locus specific | 0.192 | 0 | 0.046 | 716 | |
| vWA | Allele | No | Locus specific | 0.192 | 0.0109 | -0.12 | 25.1 | |
| D16S539 | Allele | No | Locus specific | 0.192 | 0.0114 | -0.0785 | 415 | |
| CSF1PO | Allele | No | Locus specific | 0.192 | 0.00855 | -0.0411 | 37.1 | |
| TPOX | LUS | No | Locus specific | 0.192 | 0.00573 | -0.0294 | 44.9 | |
| D8S1179 | Multi-seq | No | Locus specific | 0.192 | 0.0125 | 0.0503 | 674 | 12 |
| D21S11 | LUS | No | Locus specific | 0.192 | 0.00636 | -0.0122 | 407 | |
| D18S51 | Allele | No | Locus specific | 0.192 | 0.00657 | -0.032 | 47.3 | |
| D2S441 | LUS | No | Locus specific | 0.192 | 0.00589 | -0.0235 | 357 | |
| D19S433 | Allele | No | Locus specific | 0.192 | 0.00696 | -0.0345 | 25.2 | |
| TH01 | Allele | No | Locus specific | 0.192 | 0.00269 | -0.0032 | 434 | |
| FGA | Allele | No | Locus specific | 0.192 | 0.00697 | -0.0871 | 18.5 | |
| D22S1045 | Allele | No | Locus specific | 0.192 | 0.00793 | -0.0673 | 596 | |
| D5S818 | LUS | No | Locus specific | 0.192 | 0.00914 | -0.0474 | 27.3 | |
| D13S317 | LUS | No | Locus specific | 0.192 | 0.0102 | -0.0623 | 104 | |
| D7S820 | Multi-seq | No | Locus specific | 0.192 | 0.00538 | 0.0239 | 422 | 9 |
| SE33 | Multi-seq | No | Locus specific | 0.192 | 0.00493 | 0.0445 | 133 | 9 |
| D10S1248 | Multi-seq | No | Locus specific | 0.192 | 0.0343 | 0.0415 | 592 | 15 |
| D1S1656 | Multi-seq | No | Locus specific | 0.192 | 0.037 | 0.061 | 268 | 15 |
| D12S391 | Multi-seq | No | Locus specific | 0.192 | 0.0393 | 0.0476 | 415 | 12 |
| D2S1338 | Allele | No | Locus specific | 0.192 | 0.00472 | -0.033 | 348 | |

3. Enter names for "Parameters for Monte Carlo simulation" and "Allele repeat correction".



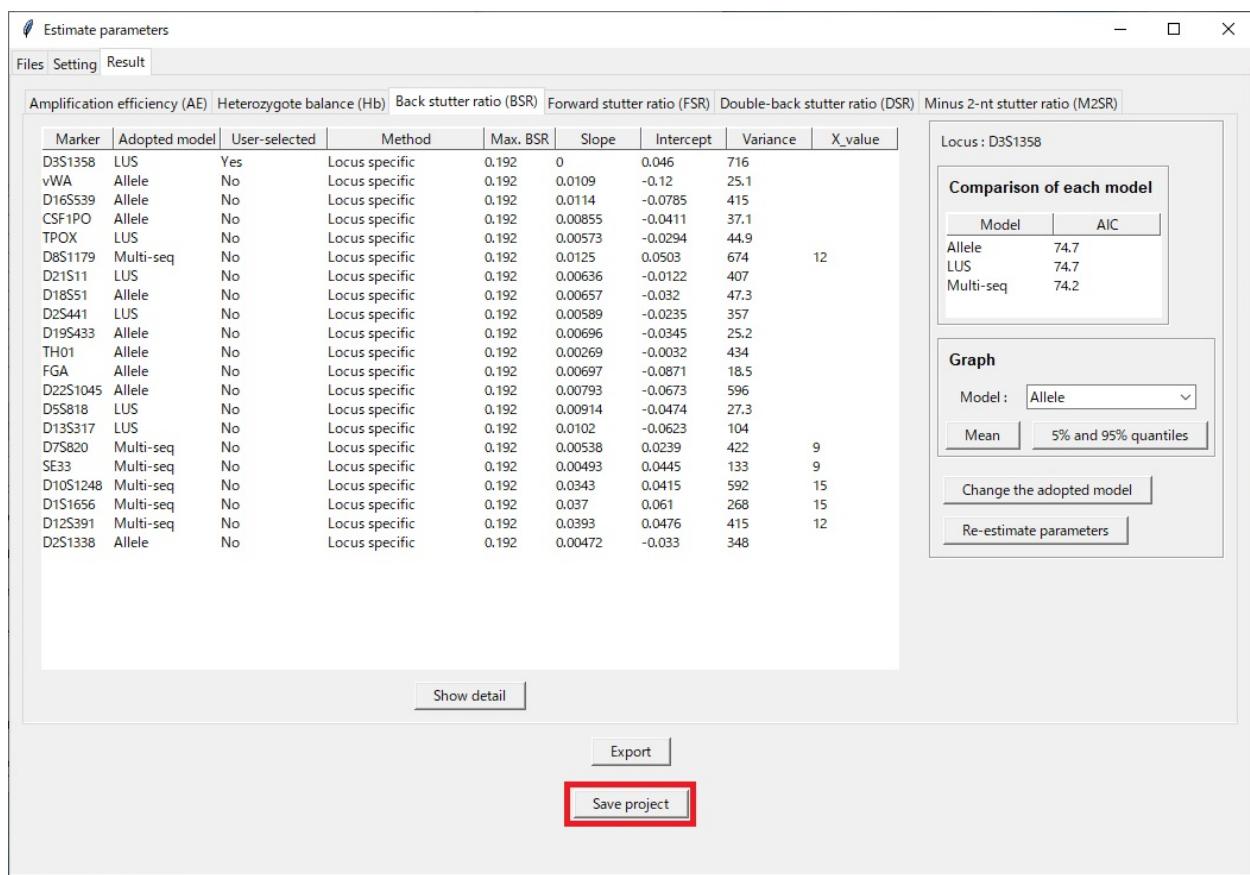
4. Click the "Save" button.



Note

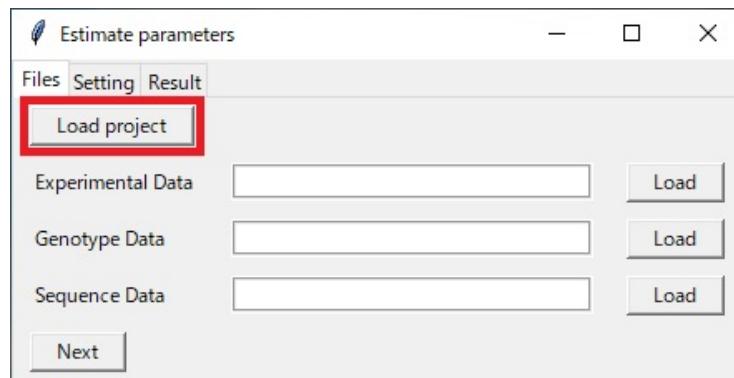
Two exported files can be directly used for setting analysis methods of "Parameters for Monte Carlo simulation" and "Allele repeat correction".

5. Click the "Save project" button to save the current project.



Note

Users can load the saved project by clicking the "Load project" button in the "Files" tab.

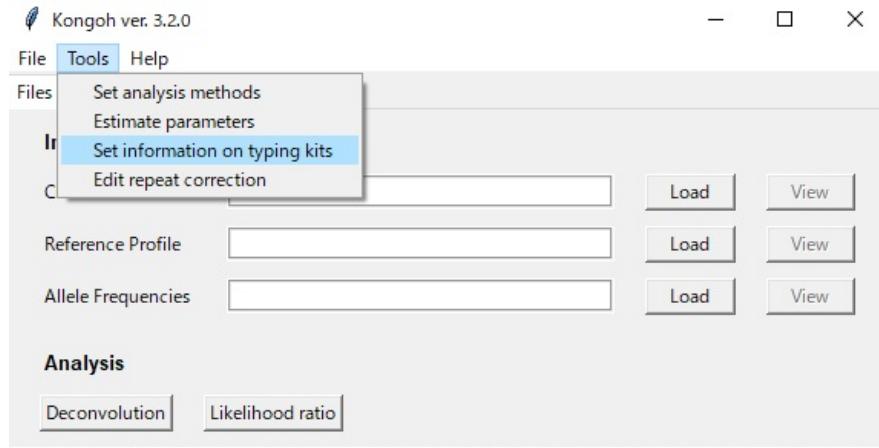


Set information on typing kits

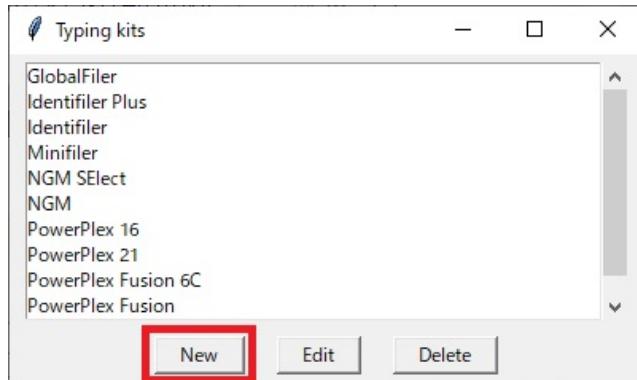
Users can create or edit information on typing kits. The information is essential to perform deconvolution and the assignment of likelihood ratios.

Create information on a typing kit

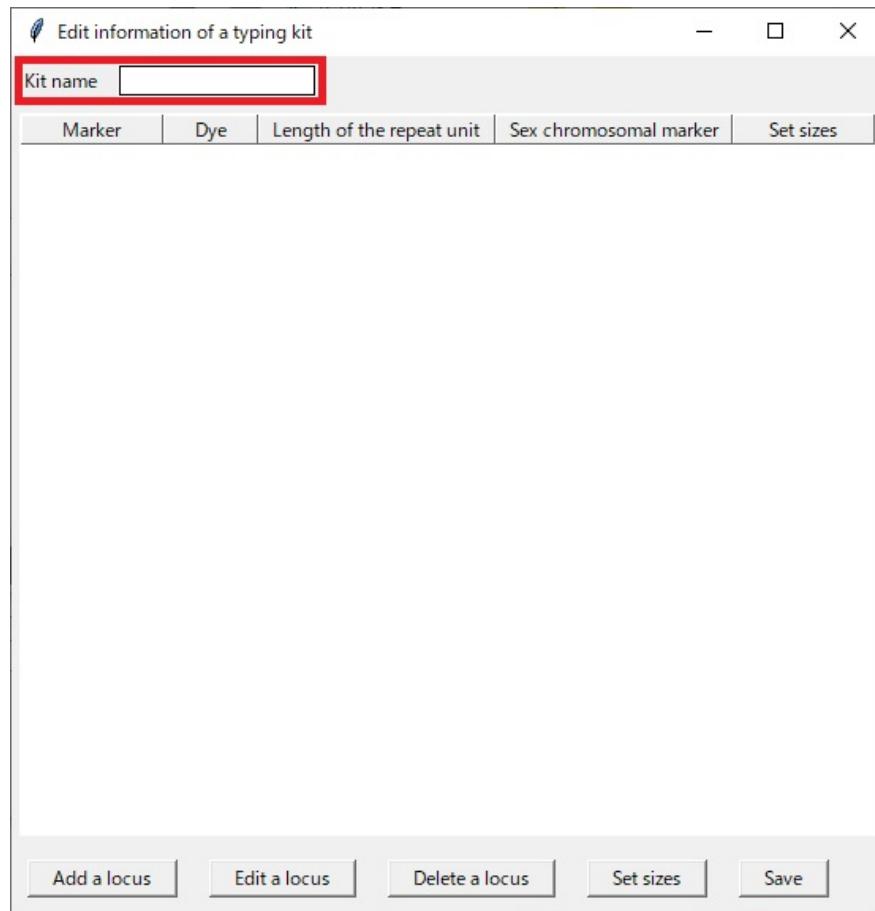
1. Go to Tools > Set information on typing kits.



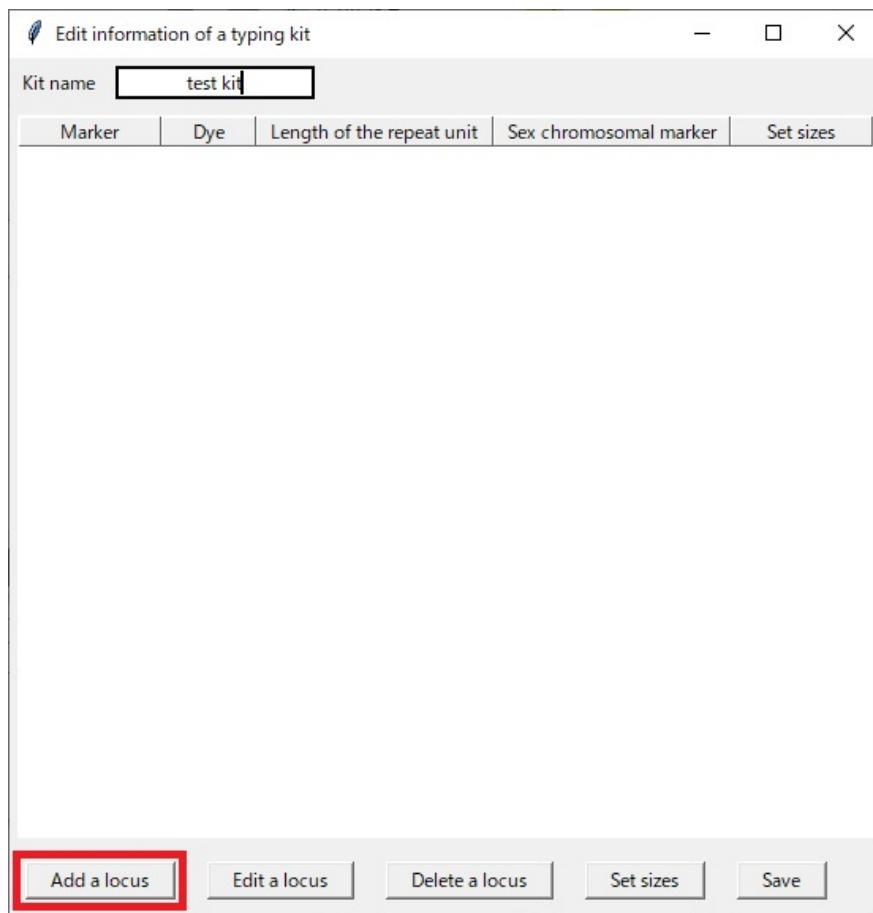
2. Click the "New" button.



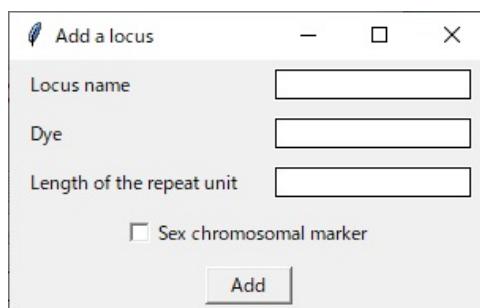
3. Enter a kit name.



4. Click the "Add a locus" button.



Then, a new window will be automatically opened.



5. Enter a locus name, the dye information, and the length of the repeat unit¹¹.

The dialog box has a title bar 'Add a locus' with minimize, maximize, and close buttons. It contains three input fields: 'Locus name' (empty), 'Dye' (empty), and 'Length of the repeat unit' (empty). Below these is a checkbox labeled 'Sex chromosomal marker' which is unchecked. At the bottom is a 'Add' button.

6. If the locus is a sex chromosomal marker, Check the "Sex chromosomal marker".

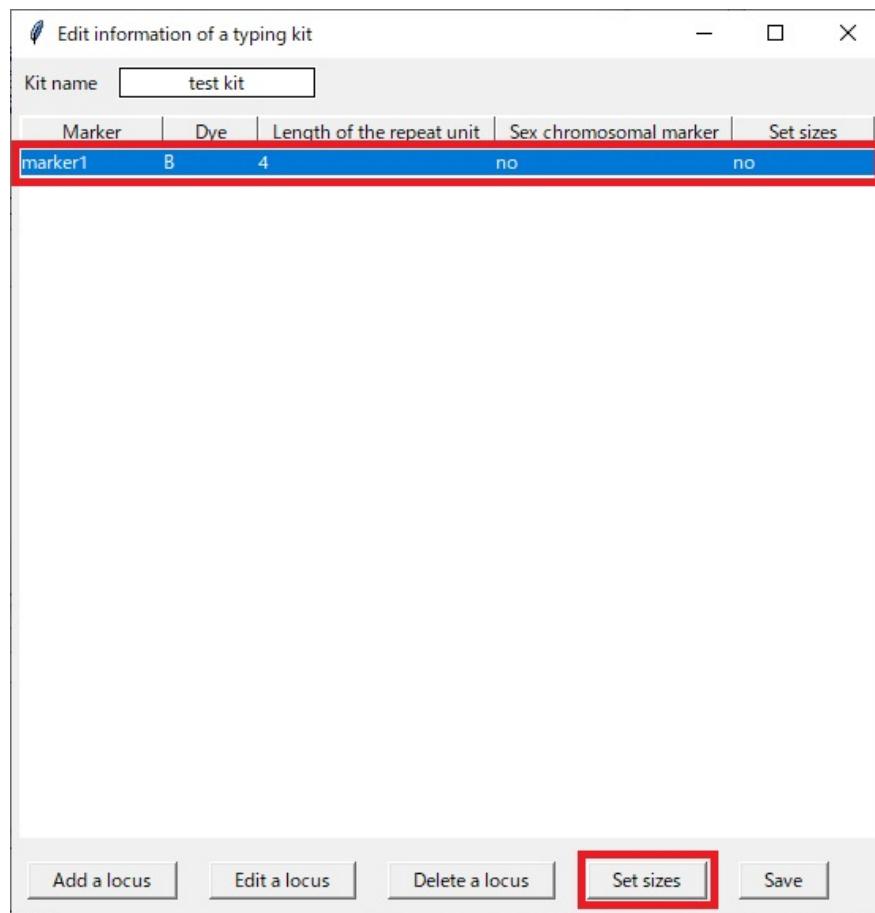
The dialog box shows the same fields as the previous screenshot. The 'Length of the repeat unit' field now contains the value '4'. The 'Sex chromosomal marker' checkbox is also highlighted with a red border.

7. Click the "Add" button. Then, the information on the locus will be displayed.

The dialog box shows the same fields as the previous screenshots. The 'Add' button at the bottom is highlighted with a red border.

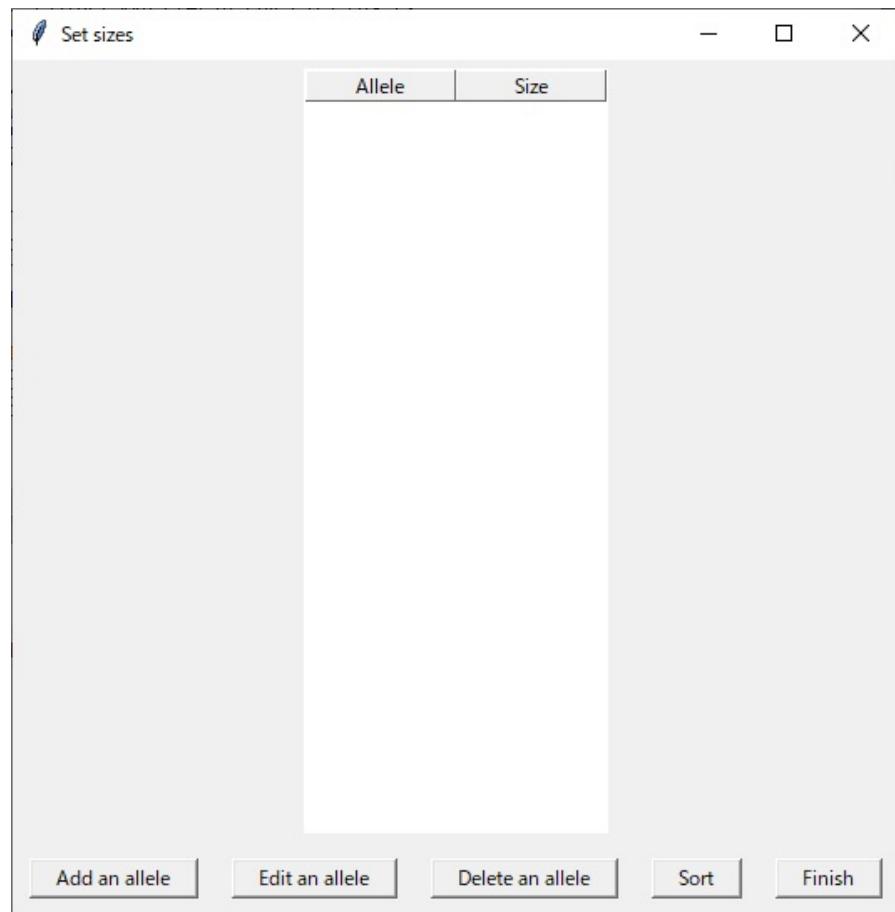
¹¹Kongoh is only applicable to the STR typing kit; therefore, the length of the repeat unit must be set in all loci other than sex chromosomal markers.

8. Select a locus and click the "Set sizes" button to set the sizes of each allele¹².

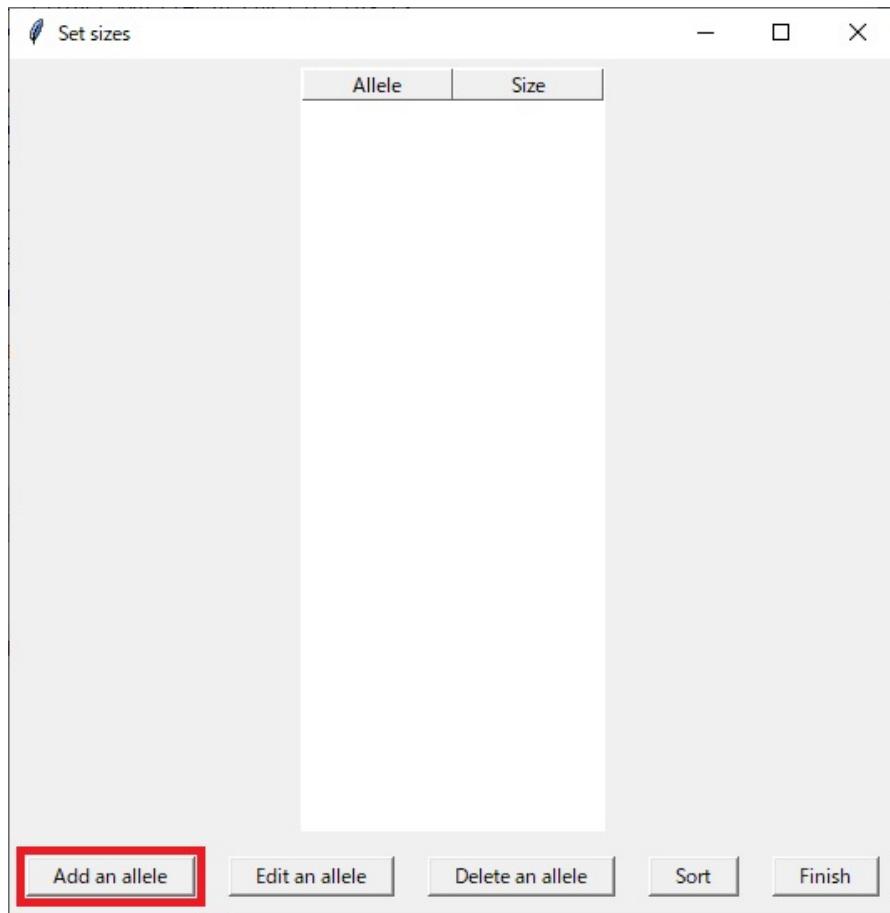


¹²The information on the sizes is used to estimate the size of a drop-out allele in the case of locus drop-out when performing deconvolution and assigning likelihood ratios.

Then, a new window will be automatically opened.



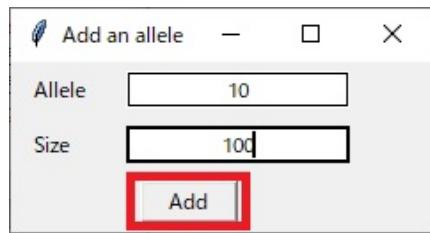
9. Click the "Add an allele" button.



10. Enter an allele name, and the size.



11. Click the "Add" button.



Then, the information on the allele and the size will be displayed.

| Set sizes | |
|-----------|------|
| Allele | Size |
| 10 | 100 |
| | |

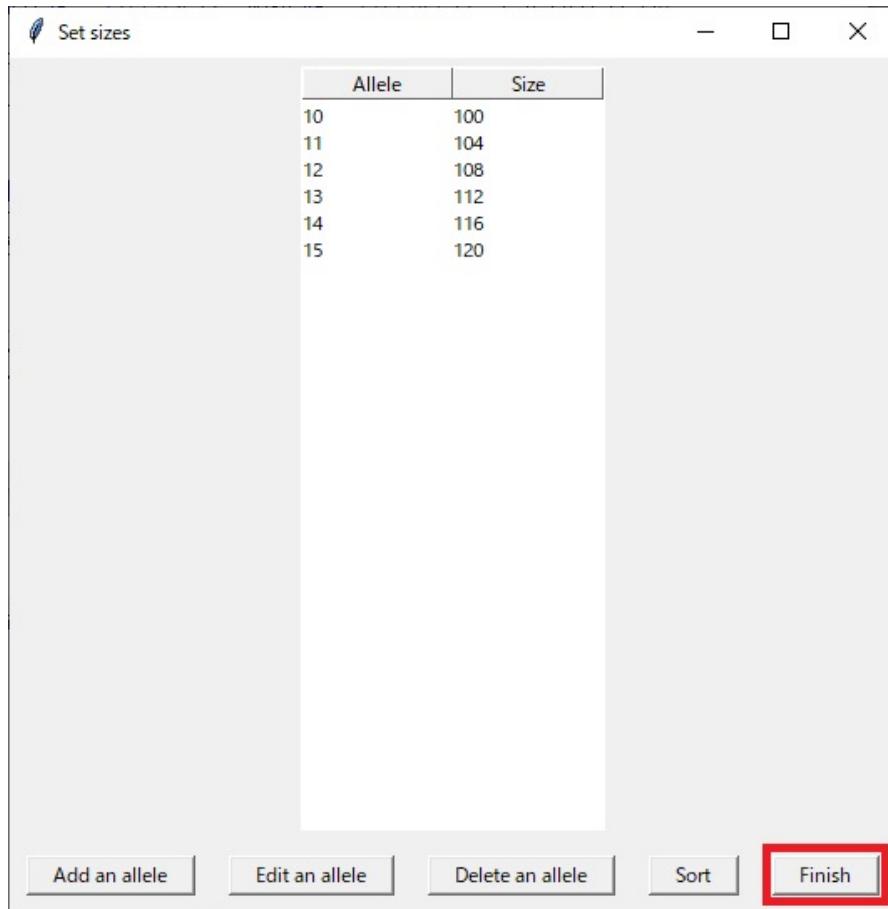
At the bottom of the window are five buttons: "Add an allele", "Edit an allele", "Delete an allele", "Sort", and "Finish". The "Add an allele" button is highlighted with a red border.

12. Repeat the addition of alleles and the sizes as many as possible.

Note

Users can edit an allele and the size from the "Edit an allele" button. Users can also delete an allele and the size from the "Delete an allele" button. Alleles can be sorted from the "Sort" button.

13. Click the "Finish" button.



Then, the "Set sizes" column will be changed to "yes"¹³.

Edit information of a typing kit

| Marker | Dye | Length of the repeat unit | Sex chromosomal marker | Set sizes |
|---------|-----|---------------------------|------------------------|-----------|
| marker1 | B | 4 | no | yes |

Add a locus Edit a locus Delete a locus Set sizes Save

¹³Users must set sizes of all loci.

14. Click the "Save" button after setting all markers.

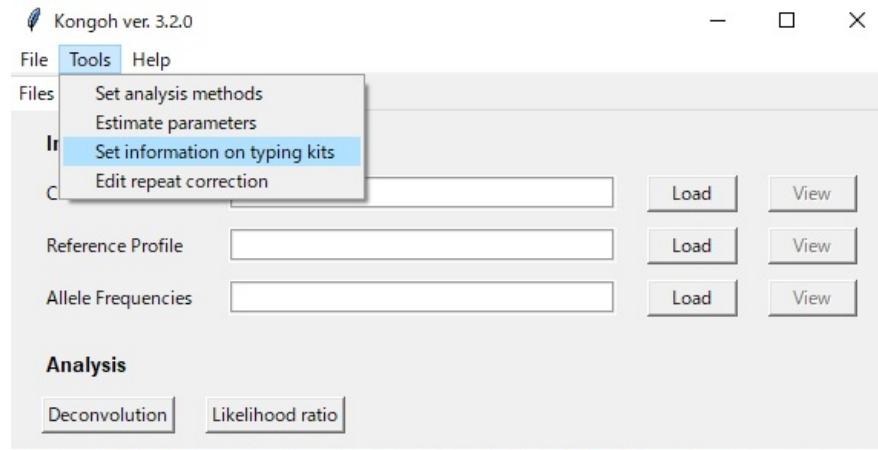
Edit information of a typing kit

| Marker | Dye | Length of the repeat unit | Sex chromosomal marker | Set sizes |
|---------|-----|---------------------------|------------------------|-----------|
| marker1 | B | 4 | no | yes |
| marker2 | B | 4 | no | yes |
| marker3 | G | 4 | no | yes |
| marker4 | G | 3 | no | yes |
| marker5 | Y | 4 | no | yes |

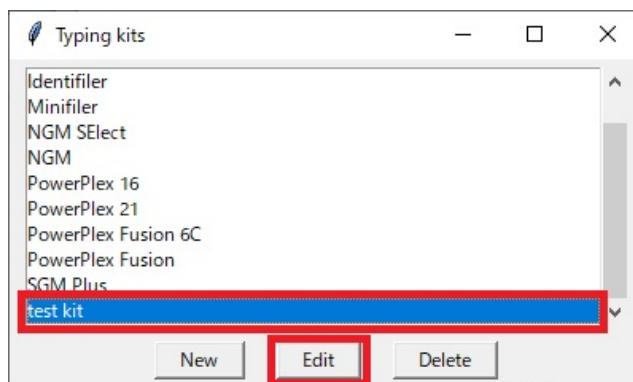
Add a locus Edit a locus Delete a locus Set sizes **Save**

Edit information on a typing kit

1. Go to Tools > Set information on typing kits.



2. Select a kit and click the "Edit" button.



3. Edit the information on the selected kit just like creating information on a typing kit.

4. Click the "Save" button.

Edit information of a typing kit

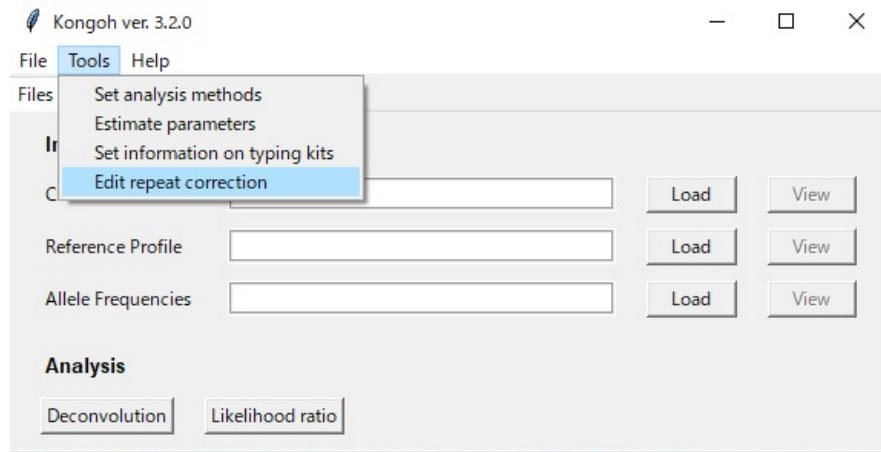
| Marker | Dye | Length of the repeat unit | Sex chromosomal marker | Set sizes |
|---------|-----|---------------------------|------------------------|-----------|
| marker1 | B | 4 | no | yes |
| marker2 | B | 4 | no | yes |
| marker3 | G | 4 | no | yes |
| marker4 | G | 3 | no | yes |
| marker5 | Y | 4 | no | yes |

Add a locus Edit a locus Delete a locus Set sizes **Save**

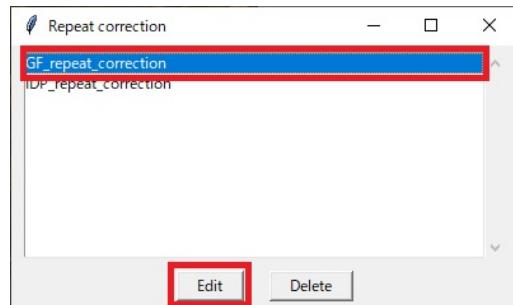
Edit repeat correction

Users can edit LUS, CA_BSR, CA_FSR, and CA_DSR. LUS is the abbreviation of the longest uninterrupted stretch[11], which is used in the LUS model for stutter ratios. CA_BSR, CA_FSR, and CA_DSR are corrected allele numbers used in the multi-sequence model for back stutter ratios, forward stutter ratios, and double-back stutter ratios, respectively.

1. Go to Tools > Edit repeat correction.



2. Select a dataset of allele repeat correction and click the "Edit" button.



3. Select a locus.

| Allele | LUS | CA_BSR | CA_FSR | CA_DSR |
|--------|-----------|--------|--------|--------|
| 11 | 8.000000 | | | |
| 12 | 10.000000 | | | |
| 13 | 10.333333 | | | |
| 14 | 11.154696 | | | |
| 15 | 12.213946 | | | |
| 15.2 | 11.000000 | | | |
| 16 | 12.947099 | | | |
| 17 | 13.586288 | | | |
| 18 | 14.105023 | | | |
| 19 | 15.000000 | | | |
| 20 | 16.500000 | | | |
| 21 | 16.996918 | | | |

Add an allele **Edit an allele** **Delete an allele** **Finish**

4. Add or edit LUS, CA_BSR, CA_FSR, or CA_DSR from the "Add an allele" button or the "Edit an allele" buttons, respectively.

| Allele | LUS | CA_BSR | CA_FSR | CA_DSR |
|--------|-----------|--------|--------|--------|
| 11 | 8.000000 | | | |
| 12 | 10.000000 | | | |
| 13 | 10.333333 | | | |
| 14 | 11.154696 | | | |
| 15 | 12.213946 | | | |
| 15.2 | 11.000000 | | | |
| 16 | 12.947099 | | | |
| 17 | 13.586288 | | | |
| 18 | 14.105023 | | | |
| 19 | 15.000000 | | | |
| 20 | 16.500000 | | | |
| 21 | 16.996918 | | | |
| 22 | 18 | | | |

Add an allele **Edit an allele** **Delete an allele** **Finish**

5. Enter the name of an allele.

The screenshot shows a Windows-style dialog box titled "Add an allele". At the top, it says "Locus D3S1358". Below that is a table with five rows. The first row has "Allele" in the left column and an empty input field in the right column, which is highlighted with a red box. The other four rows have empty input fields in both columns. At the bottom right is a "Save" button.

| | |
|--------|----------------------|
| Locus | D3S1358 |
| Allele | <input type="text"/> |
| LUS | <input type="text"/> |
| CA_BSR | <input type="text"/> |
| CA_FSR | <input type="text"/> |
| CA_DSR | <input type="text"/> |

Save

6. Enter values of LUS, CA_BSR, CA_FSR, or CA_DSR as needed.

This screenshot is similar to the previous one, but the "LUS" row in the table is now highlighted with a red box. The "Allele" field still contains "22".

| | |
|--------|-------------------------|
| Locus | D3S1358 |
| Allele | <input type="text"/> 22 |
| LUS | <input type="text"/> |
| CA_BSR | <input type="text"/> |
| CA_FSR | <input type="text"/> |
| CA_DSR | <input type="text"/> |

Save

7. Click the "Save" button.

This screenshot shows the dialog box with all fields filled: "Allele" is "22", "LUS" is "18", and the other three rows are empty. The "Save" button at the bottom right is highlighted with a red box.

| | |
|--------|-------------------------|
| Locus | D3S1358 |
| Allele | <input type="text"/> 22 |
| LUS | <input type="text"/> 18 |
| CA_BSR | <input type="text"/> |
| CA_FSR | <input type="text"/> |
| CA_DSR | <input type="text"/> |

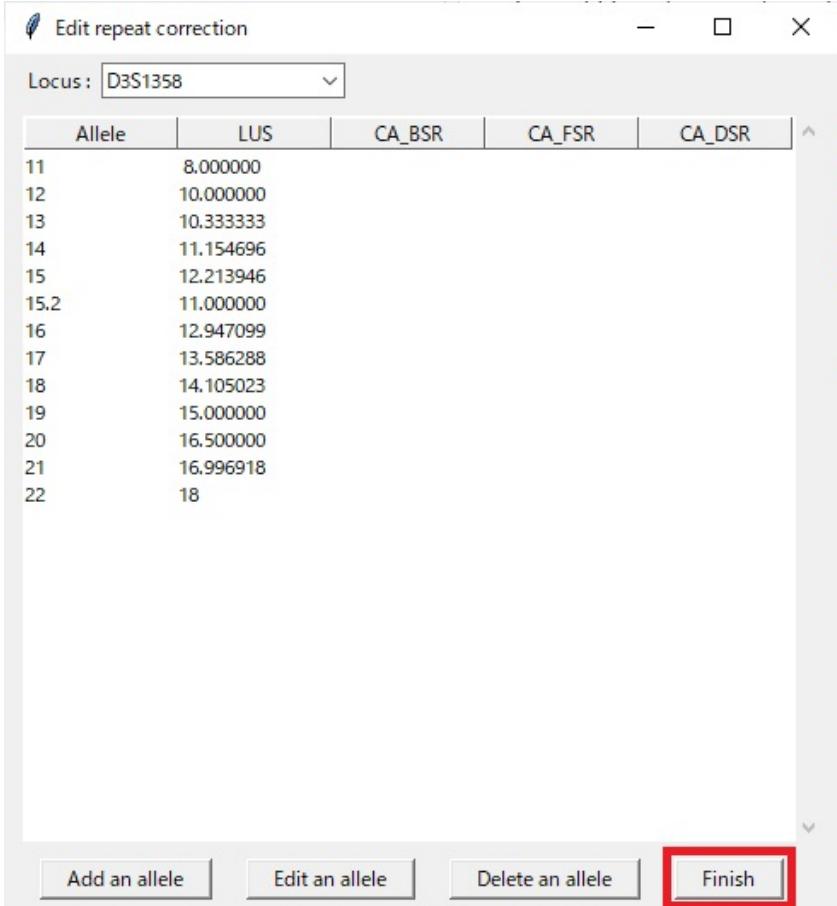
Save

8. Repeat the edit of LUS, CA_BSR, CA_FSR, and CA_DSR.

Note

Users can delete an allele from the "Delete an allele" button.

9. Click the "Finish" button after finishing all the settings.

 Edit repeat correction

Locus: D3S1358

| Allele | LUS | CA_BSR | CA_FSR | CA_DSR |
|--------|-----------|--------|--------|--------|
| 11 | 8.000000 | | | |
| 12 | 10.000000 | | | |
| 13 | 10.333333 | | | |
| 14 | 11.154696 | | | |
| 15 | 12.213946 | | | |
| 15.2 | 11.000000 | | | |
| 16 | 12.947099 | | | |
| 17 | 13.586288 | | | |
| 18 | 14.105023 | | | |
| 19 | 15.000000 | | | |
| 20 | 16.500000 | | | |
| 21 | 16.996918 | | | |
| 22 | 18 | | | |

Add an allele Edit an allele Delete an allele **Finish**

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