

RelSearch version 1.0.0

User Manual

Sho Manabe

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Chapter 1

Initial setup

1. Ensure that R ($\geq 4.4.0$) is installed. It is available from the [R Development Core Team website](#).
2. Begin an R session.
3. Execute the following command in R to install required packages.

```
install.packages('https://github.com/manabe0322/RelSearch/releases/download/
  ↵ v1.0.0/RelSearch_1.0.0.zip', repos = NULL, type = 'win.binary')
install.packages(c("shiny", "data.table", "dplyr", "DT", "magrittr",
  ↵ "pedtools", "Rcpp", "ribd", "shinyFeedback", "shinyjs", "shinythemes",
  ↵ "waiter"))
```

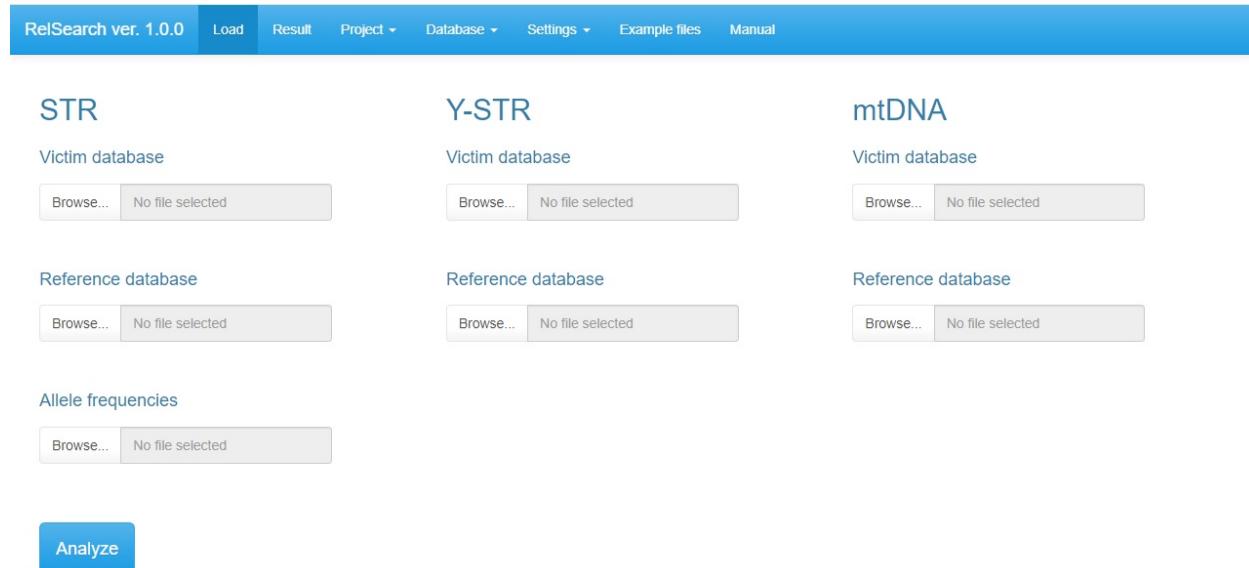
Chapter 2

Quick guide

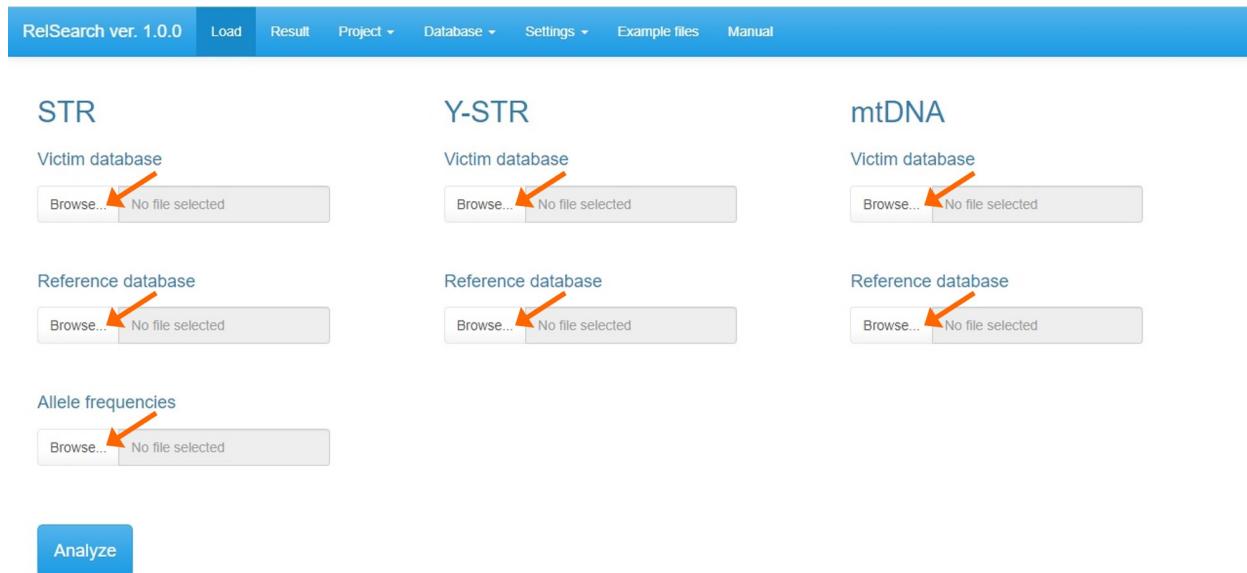
1. Execute the following commands in R to launch the software interface.

```
library(ReISearch)  
ReISearch()
```

Then, the software interface will open automatically.



2. Load files from each “**Browse...**” button.



Note

- RelSearch can analyze data for each marker type separately as well as for all marker types together.
- Victim, reference, and allele frequency databases are required to analyze autosomal STR profiles.
- Victim and reference databases are required to analyze Y-STR profiles.
- Victim and reference databases are required to analyze mtDNA profiles.
- See section [Files](#) for information on each file.

3. Click the “Analysis” button.

STR

Victim database

Browse... str_victim_example.csv
Upload complete

Reference database

Browse... str_reference_example.csv
Upload complete

Allele frequencies

Browse... str_allele-freq_example.csv
Upload complete

Analyze

Y-STR

Victim database

Browse... y_victim_example.csv
Upload complete

Reference database

Browse... y_reference_example.csv
Upload complete

mtDNA

Victim database

Browse... mt_victim_example.csv
Upload complete

Reference database

Browse... mt_reference_example.csv
Upload complete

4. After finishing the analysis, the result window will open automatically.

Result

Information

Data that satisfies the criterion of the minimum LR is displayed.

Victim	Reference	Family	Assumed relationship	LR	Estimated relationship	Estimated sex (Victim)	Estimated sex (Reference)	Paternal lineage	Maternal lineage	Search:
All	All	All	All	All	All	All	All	All	All	
V4	R4	F4	Mother_Daughter	6.33e+9	Mother_Daughter					Support
V2	R2	F2	Father_Daughter	4.50e+8	Father_Daughter	Male				Not support
V3	R3	F3	Mother_Son	9.01e+7	Mother_Son					Support
V1	R1	F1	Father_Son	4.12e+6	Father_Son	Male	Male	Support		Not support
V19	R19	F19	Father_Daughter	3.93e+6	Father_Daughter	Male				Not support
V5	R5	F5	Brother_Brother	2.48e+5	Brother_Brother	Male	Male	Support	Support	
V7	R7	F7	Sister_Brother	5.90e+4	Sister_Brother					Support
V22	R22	F21	Brother_Sister	1.77e+4		Male				Not support
V21	R21	F20	Paternal-aunt_Nephew	7.95e+3	Paternal-aunt_Nephew					Not support
V21	R21	F20	Paternal-aunt_Nephew	7.95e+3	Paternal-aunt_Nephew	Male				Not support

Show 10 entries

Display setting

Default display

Identified

Inconclusive

Excluded by Y-STR or mtDNA

Minimum LR displayed

100

Apply

Download

Information

Previous 1 2 3 Next

Note

- See section **Result** for navigation of the result window.

5. Select Project ► Save project.

RelSearch ver. 1.0.0 Load Result Project Database Settings Example files Manual

Result

New project
Load project
Save project

Show 10 entries

Victim	Reference	Family	Assumed relationship	LR	Estimated relationship	Estimated sex (Victim)	Estimated sex (Reference)	Paternal lineage	Maternal lineage
V4	R4	F4	Mother_Daughter	6.33e+9	Mother_Daughter			Support	
V2	R2	F2	Father_Daughter	4.50e+8	Father_Daughter	Male		Not support	
V3	R3	F3	Mother_Son	9.01e+7	Mother_Son		Male	Support	
V1	R1	F1	Father_Son	4.12e+6	Father_Son	Male	Male	Support	Not support
V19	R19	F19	Father_Daughter	3.93e+6	Father_Daughter	Male		Not support	
V5	R5	F5	Brother_Brother	2.48e+5	Brother_Brother	Male	Male	Support	Support
V7	R7	F7	Sister_Brother	5.90e+4	Sister_Brother		Male	Support	
V22	R22	F21	Brother_Sister	1.77e+4		Male		Not support	
V21	R21	F20	Paternal-aunt_Nephew	7.95e+3	Paternal-aunt_Nephew		Male	Not support	
V21	R21	F20	Paternal-aunt_Nephew	7.95e+3	Paternal-aunt_Nephew		Male	Not support	

Showing 1 to 10 of 21 entries

Previous 2 3 Next

Display setting

Default display

Identified

Inconclusive

Excluded by Y-STR or mtDNA

Minimum LR displayed

100

Apply

Download

6. Enter the project name and click the “**Save as**” button.



Note

- The saved project can be loaded from **Project ▶ Load project**.
- How to handle projects is described in section **Other functions**.

Chapter 3

Files

3.1 STR: Victim database

SampleName	D3S1358	D3S1358	vWA	vWA	D16S539	D16S539	CSF1PO	CSF1PO
Victim1	17	17	17		11		12	
Victim2	17		18	18			10	11
Victim3	15	17	17	18	10	10	10	12
Victim4	15	18	15	18	9	12	11	12
Victim5	15	15	18		9	9	10	

Note

- File type: .csv
- This file requires the column “SampleName” and columns for each marker (two columns in each).
- The marker with two empty cells (e.g., D16S539 of the sample “Victim2” in the above table) is ignored when calculating the likelihood ratio.
- The marker with one empty cell (e.g., vWA of the sample “Victim1” in the above table) can be regarded as both homozygote (i.e., no drop-out) and heterozygote with drop-out of one allele when calculating the likelihood ratio.

3.2 STR: Reference database

SampleName	Family	Relationship	D3S1358	D3S1358	vWA	vWA	D16S539	D16S539
Reference1	Family 1	Father_Son	17	17	14	17	10	10
Reference2	Family 2	Brother_Brother	16	17	17	17	10	12
Reference3	Family 3	Brother_Brother	16	17	14	18	9	9
Reference3	Family 3	Father_Son	16	17	14	18	9	9
Reference4	Family 4	Brother_Brother	15	16	17		9	12
Reference5	Family 4	Uncle_Nephew	15	15	14	16		

Note

- File type: .csv
- This file requires the columns “SampleName”, “Family”, “Relationship”, and columns for each marker (two columns in each).
- The names of the relationship need to be pre-defined in **Settings ▶ Relationships** (see section [Setting](#)).
- If there are multiple missing family members for a reference, add rows for each relationship of the members (e.g., Brother_Brother and Father_Son of the sample “Reference3” in the above table).
- The marker with two empty cells (e.g., D16S539 of the sample “Reference5” in the above table) is ignored when calculating the likelihood ratio.
- The marker with one empty cell (e.g., vWA of the sample “Reference4” in the above table) can be regarded as both homozygote (i.e., no drop-out) and heterozygote with drop-out of one allele when calculating the likelihood ratio.

3.3 STR: Allele frequencies

Allele	D3S1358	vWA	D16S539	CSF1PO	TPOX
10			602	648	100
11			562	620	1072
12	6		517	1267	114
13	3	1	209	208	3
14	79	586	26	52	2
15	1192	78	3	14	

Note

- File type: .csv
- This file requires the columns “Allele” and columns for each marker (one column in each).
- Allele counts in the population database are entered in the columns for each marker.

3.4 Y-STR: Victim database

SampleName	DYS392	DYS518	DYS570	DYS437	DYS385
Victim1	11	37	17	14	13,17
Victim2	13	38	19	14	10,20
Victim3	11	37	17	14	14
Victim4	11	37	16	14	13
Victim5			18		13,17

Note

- File type: .csv
- This file requires the column “SampleName” and columns for each marker (one column in each).
- In the marker with more than one allele, each allele must be separated by a comma without any spaces (e.g., DYS385 in the above table).
- The marker with an empty cell (e.g., DYS392 of the sample ‘Victim5’ in the above table) is ignored for the analysis.

3.5 Y-STR: Reference database

SampleName	Family	Relationship	DYS392	DYS518	DYS570	DYS437	DYS385
Reference1	Family 1	Father_Son	13	38	19	14	10,20
Reference2	Family 2	Brother_Brother	11	37	17	14	14,17
Reference3	Family 3	Brother_Brother	11	38	16	14	11
Reference3	Family 3	Father_Son	11	38	16	14	11
Reference4	Family 4	Brother_Brother	11	38	19	14	13,15
Reference5	Family 4	Uncle_Nephew	14		18	14	13,18

Note

- File type: .csv
- This file requires the column "SampleName", "Family", "Relationship", and columns for each marker (one column in each).
- The names of the relationship need to be pre-defined in **Settings ▶ Relationships** (see section [Setting](#)).
- If there are multiple missing family members for a reference, add rows for each relationship of the members (e.g., Brother_Brother and Father_Son of the sample "Reference3" in the above table).
- In the marker with more than one allele, each allele must be separated by a comma without any spaces (e.g., DYS385 in the above table).
- The marker with an empty cell (e.g., DYS518 of the sample 'Reference5' in the above table) is ignored for the analysis.

3.6 mtDNA: Victim database

SampleName	Range	Haplotype
Victim1	73-340 16024-16365	16183C 16189C 16217C 16311C 73G 263G 309.1C 315.1C
Victim2	73-340 16024-16365	16093C 16114A 16223T 16362C 73G 263G 309.1C 315.1C
Victim3	73-167 240-340 16024-16365	16095T 16189C 16223T 16265C 16274A 16362C 73G 263G
Victim4	73-265 16024-16284	16223T 73G 152C 263G
Victim5		

Note

- File type: .csv
- This file requires the column "SampleName", "Range", and "Haplotype".
- In the 'Range' column, the first and the last positions in each readable sequence must be written with a hyphen between these positions (e.g., 73-340). The readable sequences must be separated by a single blank space.
- In the 'Haplotype' column, the mtDNA types are expressed as the differences to a reference sequence such as the revised Cambridge Reference Sequence [1] (e.g., 73G, 315.1C, and so on). Each mtDNA type must be separated by a single blank space.
- The empty cells in the 'Range' and 'Haplotype' columns (e.g., the sample 'Victim5' in the above table) mean that there is no readable sequence.

3.7 mtDNA: Reference database

SampleName	Family	Relationship	Range	Haplotype
Reference1	Family 1	Mother_Daughter	73-340 16024-16365	16223T 73G 263G 309.1C 315.1C
Reference2	Family 2	Mother_Daughter	73-340 16024-16365	16172C 16223T 73G 150T 263G
Reference3	Family 3	Brother_Brother	73-340 16024-16365	16172C 16189C 16223T 73G 263G
Reference3	Family 3	Father_Son	73-340 16024-16365	16172C 16189C 16223T 73G 263G
Reference4	Family 4	Sister_Sister	73-340 16024-16365	16223T 16362C 73G 263G 315.1C
Reference5	Family 4	Aunt_Niece		

Note

- File type: .csv
- This file requires the column "SampleName", "Family", "Relationship", "Range", and "Haplotype".
- The names of the relationship need to be pre-defined in **Settings ▶ Relationships** (see section [Setting](#)).
- If there are multiple missing family members for a reference, add rows for each relationship of the members (e.g., Brother_Brother and Father_Son of the sample "Reference3" in the above table).
- In the 'Range' column, the first and the last positions in each readable sequence must be written with a hyphen between these positions (e.g., 73-340). The readable sequences must be separated by a single blank space.
- In the 'Haplotype' column, the mtDNA types are expressed as the differences to a reference sequence such as the revised Cambridge Reference Sequence [1] (e.g., 73G, 315.1C, and so on). Each mtDNA type must be separated by a single blank space.
- The empty cells in the 'Range' and 'Haplotype' columns (e.g., the sample 'Reference5' in the above table) mean that there is no readable sequence.

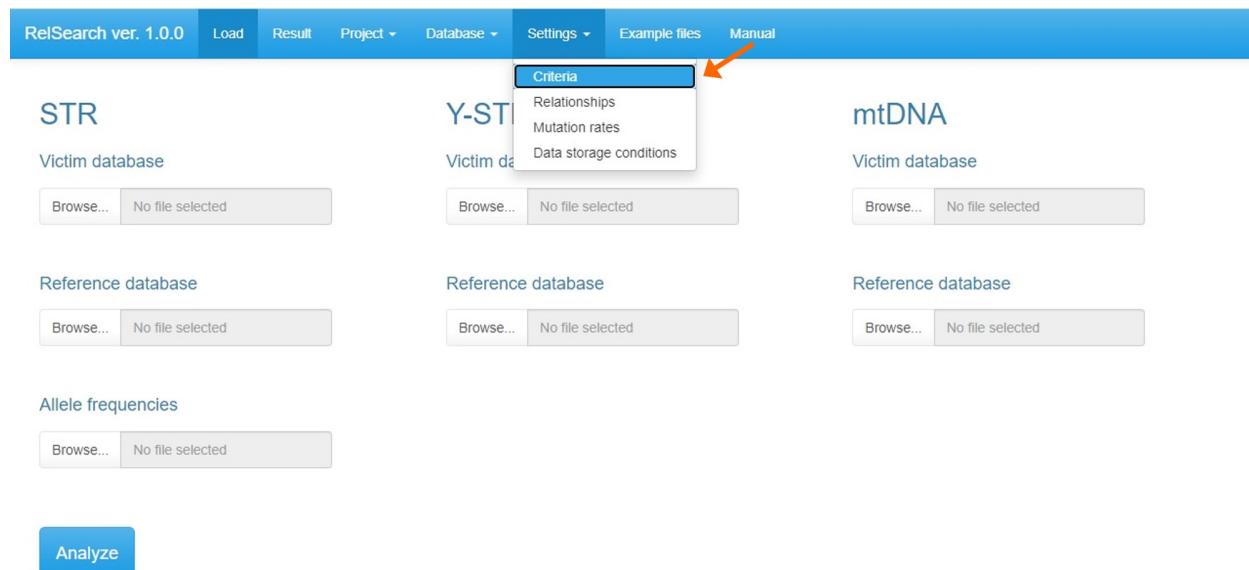
Chapter 4

Setting

4.1 Criteria

In RelSearch, there are some criteria to determine whether each victim-reference pair has an assumed relationship or not. For autosomal STR, RelSearch calculates LR values for each victim-reference pair and investigates whether these LR values are greater than an LR threshold or not. For Y-STR, RelSearch analyzes the number of mismatched loci and the total mismatched steps between victim and reference Y-STR profiles to search paternal lineages. For mtDNA, RelSearch investigates the number of mismatched nucleotides between the victim and the reference sequences to search maternal lineages.

To set criteria, select **Setting ▶ Criteria**.



Then the criteria setting window will open automatically.

The screenshot shows the RelSearch software interface with a blue header bar containing the text "RelSearch ver. 1.0.0" and several menu items: Load, Result, Project, Database, Settings (selected), Example files, and Manual. Below the header is a section titled "Criteria" with the sub-instruction "Press the save button to reflect the changes." There are three main sections: "STR", "Y-STR", and "mtDNA".

- STR:** Contains a field "Minimum LR" with the value "100".
- Y-STR:** Contains two fields: "Maximum number of mismatched loci" with the value "4" and "Maximum total mutational steps" with the value "5".
- mtDNA:** Contains a field "Maximum number of inconsistency" with the value "1".

At the bottom left are two buttons: "Save" and "Reset".

The criteria for each marker type is as follows:

STR

- Minimum LR: The minimum likelihood ratio for which the victim and reference are estimated to have the assumed relationship.

Y-STR

- Maximum number of mismatched loci: The upper limit of the number of mismatched loci between victim and reference profiles for which paternal lineage is not negated.
- Maximum total mutational steps: The upper limit of total mutational steps (i.e., sum of mutational steps of all loci) between victim and reference profiles for which paternal lineage is not negated.

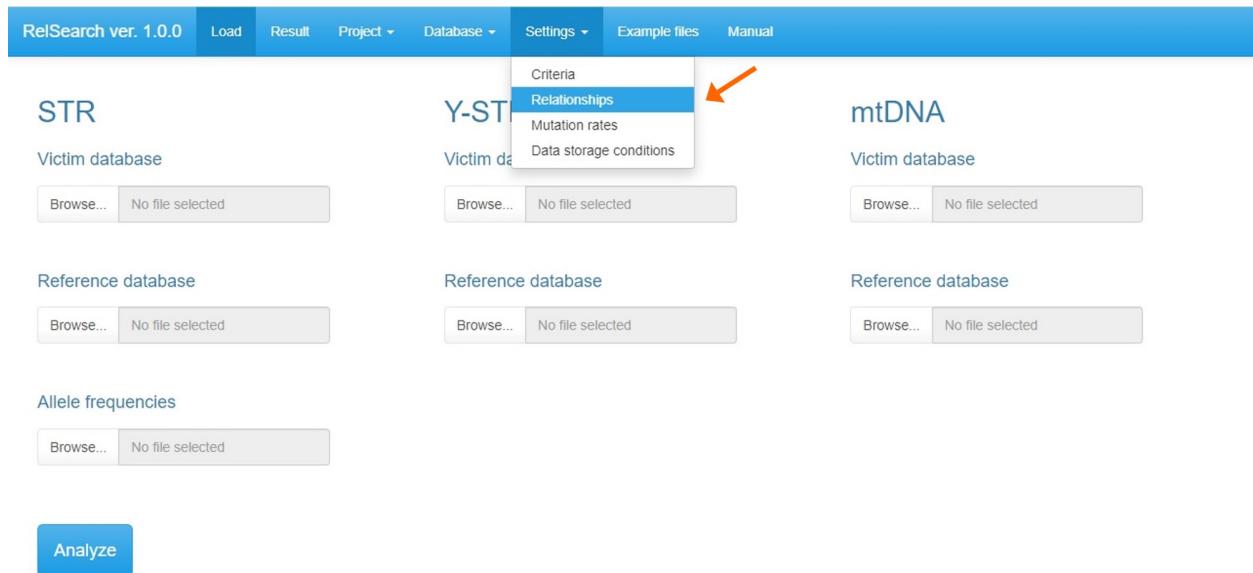
mtDNA

- Maximum number of inconsistency: The upper limit of inconsistency between victim and reference profiles for which maternal lineage is not negated.

After changing the criteria, press the **Save** button to reflect the changes. Press the **Reset** button if you want to return to default values.

4.2 Relationship

The names of the relationship in each reference profile need to be pre-defined. To define the relationship names, select **Setting ▶ Relationships**.



Then the relationship setting window will open automatically.

The screenshot shows the RelSearch software interface with a blue header bar containing the version "RelSearch ver. 1.0.0" and menu items: Load, Result, Project, Database, Settings, Example files, and Manual. Below the header is a sidebar on the left with buttons for Edit, Add, Delete, Reset, and Family tree. The main area is titled "Relationships" and contains a table with 44 rows of pre-defined relationships. The columns are: Relationship, Sex (Victim), Sex (Reference), Pr (IBD = 2), Pr (IBD = 1), Pr (IBD = 0), Paternal lineage, and Maternal lineage. The table includes entries like Father_Son, Father_Daughter, Mother_Son, etc. At the bottom of the table, it says "Showing 1 to 10 of 44 entries" and has navigation buttons for Previous, Next, and page numbers 1, 2, 3, 4, 5.

Relationships							
Show 10 entries							
Relationship	Sex (Victim)	Sex (Reference)	Pr (IBD = 2)	Pr (IBD = 1)	Pr (IBD = 0)	Paternal lineage	Maternal lineage
Father_Son	M	M	0	1	0	Yes	No
Father_Daughter	M	F	0	1	0	No	No
Mother_Son	F	M	0	1	0	No	Yes
Mother_Daughter	F	F	0	1	0	No	Yes
Son_Father	M	M	0	1	0	Yes	No
Son_Mother	M	F	0	1	0	No	Yes
Daughter_Father	F	M	0	1	0	No	No
Daughter_Mother	F	F	0	1	0	No	Yes
Brother_Brother	M	M	0.25	0.5	0.25	Yes	Yes
Brother_Sister	M	F	0.25	0.5	0.25	No	Yes

There are 44 pre-defined victim-reference relationships as the default. The names of these relationships are determined in the order of victim_reference (e.g., Father_Son means that a victim is the father and a reference is the son). You are free to change these names.

Information on the table is as follows.

- **Sex (Victim):** The biological sex of the victim
- **Sex (Reference):** The biological sex of the reference
- **Pr(IBD = 2):** The probability that two alleles are identity by descent (IBD).
- **Pr(IBD = 1):** The probability that one allele is identity by descent (IBD).
- **Pr(IBD = 0):** The probability that zero allele is identity by descent (IBD).
- **Paternal lineage:** Information on whether each relationship is paternal lineage or not.
- **Maternal lineage:** Information on whether each relationship is maternal lineage or not.

You can edit, add, and delete the relationships from the left sidebar.

4.2.1 Edit

You can edit the relationship names from the **Edit** button.

The screenshot shows the RelSearch version 1.0.0 software interface. The main window has a dark blue header with menu items: Load, Result, Project, Database, Settings, Example files, and Manual. On the left, there's a sidebar with buttons for Edit, Add, Delete, Reset, and Family tree. The main content area is titled 'Relationships' and contains a table of relationships. A pop-up window titled 'Edit the name of the relationship' is overlaid on the screen. It has a dropdown menu 'Select a relationship' containing 'Father_Son', and a text input field 'Enter a new name' which is currently empty. At the bottom of the pop-up are 'Save' and 'Cancel' buttons. Below the pop-up, the main table is visible, showing columns for relationship names, gender (M/F), counts (0-1), proportions (0-0.5), and lineage (Yes/No). The table includes entries like Son_Father, Son_Mother, Daughter_Father, Daughter_Mother, Brother_Brother, and Brother_Sister. The bottom of the main window shows pagination from 1 to 5.

Mother_Daughter	Father_Son	Gender	Count	Proportion	Lineage	Maternal lineage
Son_Father	M	M	0	1	0 Yes	No
Son_Mother	M	F	0	1	0 No	Yes
Daughter_Father	F	M	0	1	0 No	No
Daughter_Mother	F	F	0	1	0 No	Yes
Brother_Brother	M	M	0.25	0.5	0.25 Yes	Yes
Brother_Sister	M	F	0.25	0.5	0.25 No	Yes

In the pop-up window, select a relationship from the defined relationships, and enter a new name of the selected relationship. Press the **Save** button to reflect the new name.

4.2.2 Add

You can define a new relationship from the **Add** button. Then, the following pop-up window will open.

Add information on a relationship

Relationship

Family tree

Incorrect setting of the family tree!

Set a family tree

[Add a person](#)[Delete a person](#)[View family tree](#)

Person	Sex	Father	Mother	Founder
Victim	M	Victim	Victim	No
Ref	M	Victim	Victim	No

[Save](#) [Cancel](#)

The procedure is as follows.

1. Enter a relationship name.
2. Make a family tree to define the relationship between a victim and a reference. The following is a list of functions to make a family tree.
 - **Add a person:** The function to add an unknown person (UK) to the list of persons.
 - **Delete a person:** The function to delete an unknown person (UK) from the list of persons.
 - **Sex:** The function to select a biological sex (male: M or female: F).
 - **Father:** The function to select a person who is the father.
 - **Mother:** The function to select a person who is the mother.
 - **Founder:** The function to select whether the person is a founder of the family tree or not.

Here is an example of defining the brother-brother relationship for the victim-reference pair.

- 2.1. Press the **Add** button twice to add the untyped father and mother. UK1 and UK2 are assumed to be the father and mother, respectively.
- 2.2. Set the sex of the Victim, Ref, UK1, and UK2 to "M", "M", "M", and "F", respectively.
- 2.3. In the Founder column, select "No" for Victim and Ref, and select "Yes" for UK1 and UK2.
- 2.4. In the Father column, select "UK1" for Victim and Ref.
- 2.5. In the Mother column, select "UK2" for Victim and Ref.

Set a family tree

Add a person	Delete a person	View family tree		
Person	Sex	Father	Mother	Founder
Victim	M	UK1	UK2	No
Ref	M	UK1	UK2	No
UK1	M	Victim	Victim	Yes
UK2	F	Victim	Victim	Yes

3. Press the **View family tree** button to check whether the relationship between a victim and a reference is correctly defined or not.

Add information on a relationship

Relationship

Family tree

Incorrect setting of the family tree!

Set a family tree

Add a personDelete a personView family tree

Person	Sex	Father	Mother	Founder
Victim	M	UK1	UK2	No
Ref	M	UK1	UK2	No
UK1	M	Victim	Victim	Yes
UK2	F	Victim	Victim	Yes

Save Cancel



If the relationship between a victim and a reference is correctly defined, the family tree will be displayed.

Add information on a relationship

Relationship

Family tree

```
graph TD; UK1[UK1] --- Line1[ ]; Line1 --- UK2[UK2]; Line1 --- Ref[Ref]; Line1 --- Victim[Victim]
```

Set a family tree

[Add a person](#)

[Delete a person](#)

[View family tree](#)

Person	Sex	Father	Mother	Founder
Victim	M	UK1	UK2	No
Ref	M	UK1	UK2	No
UK1	M	Victim	Victim	Yes
UK2	F	Victim	Victim	Yes

[Save](#)

[Cancel](#)

4. Press the **Save** button.

Add information on a relationship

Relationship

Family tree

```
graph TD; UK1[UK1] --- Ref[Ref]; UK2([UK2]) --- Ref; Ref --- Victim[Victim]; Ref --- Ref2[Ref];
```

Set a family tree

[Add a person](#)[Delete a person](#)[View family tree](#)

Person	Sex	Father	Mother	Founder
Victim	M	UK1	UK2	No
Ref	M	UK1	UK2	No
UK1	M	Victim	Victim	Yes
UK2	F	Victim	Victim	Yes

[Save](#) Cancel

Note

Information on the biological sex, IBD probabilities, paternal lineage, and maternal lineage in the new relationship is automatically determined based on your-defined family tree.

4.2.3 Delete

You can delete a defined relationship from the **Delete** button.

In the pop-up window, select a relationship from the defined relationships. Press the **Save** button to delete the selected relationship.

The screenshot shows the RelSearch software interface. On the left, there's a sidebar with buttons for Edit, Add, Delete (which is highlighted), Reset, and Family tree. The main area has tabs for Relationship, Project, Database, Settings, Example files, and Manual. A modal dialog box is open in the center, titled "Delete information on a relationship". It contains a dropdown menu labeled "Select a relationship" with "Father_Son" selected. At the bottom right of the dialog are "Save" and "Cancel" buttons. Below the dialog is a table with columns: Father_Daughter, M, F, 0, 1, 0, No, and Maternal lineage (No). The table lists various relationships with their respective gender symbols (M for male, F for female) and numerical values. At the bottom of the table, it says "Showing 1 to 10 of 44 entries" and has navigation buttons for Previous, Next, and page numbers 1, 2, 3, 4, 5.

Father_Daughter	M	F	0	1	0	No	Maternal lineage
Mother_Son	F	M	0	1	0	No	Yes
Mother_Daughter	F	F	0	1	0	No	Yes
Son_Father	M	M	0	1	0	Yes	No
Son_Mother	M	F	0	1	0	No	Yes
Daughter_Father	F	M	0	1	0	No	No
Daughter_Mother	F	F	0	1	0	No	Yes
Brother_Brother	M	M	0.25	0.5	0.25	Yes	Yes
Brother_Sister	M	F	0.25	0.5	0.25	No	Yes

4.2.4 Reset

If you want to return to default settings, press the **Reset** button. In the pop-up window, press the **Restore default** button.

The screenshot shows the RelSearch version 1.0.0 interface. On the left, there's a sidebar with buttons for Edit, Add, Delete, Reset (which is highlighted), and Family tree. The main area is titled 'Relationships' and displays a table of relationships. A modal dialog box is open over the table, titled 'Reset information on the relationship'. Inside the dialog, it says 'Click Restore default to remove all changes.' At the bottom right of the dialog are 'Restore default' and 'Cancel' buttons. The table has columns for Relationship, Sexes (M/F), and various numerical and categorical values. The last row shows 'Brother_Sister' with values 0.25, 0.5, 0.25, Yes, and Yes. Below the table, it says 'Showing 1 to 10 of 44 entries' and has a page navigation section with buttons for Previous, Next, and page numbers 1 through 5.

Relationship	M	F	0	1	0	Yes	No
Father_Son	M	M	0	1	0	Yes	No
Father_Daughter	M	F	0	1	0	No	No
Mother_Son	F	M	0	1	0	No	Yes
Mother_Daughter	F	F	0	1	0	No	Yes
Son_Father	M	M	0	1	0	Yes	No
Son_Mother	M	F	0	1	0	No	Yes
Daughter_Father	F	M	0	1	0	No	No
Daughter_Mother	F	F	0	1	0	No	Yes
Brother_Brother	M	M	0.25	0.5	0.25	Yes	Yes
Brother_Sister	M	F	0.25	0.5	0.25	No	Yes

4.2.5 Family tree

You can check the family tree of each defined relationship. Select a row from the relationship table and press the **Family tree** button.

RelSearch ver. 1.0.0 Load Result Project ▾ Database ▾ Settings ▾ Example files Manual

Relationships

Relationship	Sex (Victim)	Sex (Reference)	Pr (IBD = 2)	Pr (IBD = 1)	Pr (IBD = 0)	Paternal lineage	Maternal lineage
Father_Son	M	M	0	1	0	Yes	No
Father_Daughter	M	F	0	1	0	No	No
Mother_Son	F	M	0	1	0	No	Yes
Mother_Daughter	F	F	0	1	0	No	Yes
Son_Father	M	M	0	1	0	Yes	No
Son_Mother	M	F	0	1	0	No	Yes
Daughter_Father	F	M	0	1	0	No	No
Daughter_Mother	F	F	0	1	0	No	Yes
Brother_Brother	M	M	0.25	0.5	0.25	Yes	Yes
Brother_Sister	M	F	0.25	0.5	0.25	No	Yes

Show 10 entries Search:

Showing 1 to 10 of 44 entries Previous 1 2 3 4 5 Next

Then, the family tree will be displayed.

RelSearch ver. 1.0.0 Load Result Project ▾ Database ▾ Settings ▾ Example files Manual

Relationship: Father_Son

Relationships

Relationship
Father_Son
Father_Daughter
Mother_Son
Mother_Daughter
Son_Father
Son_Mother
Daughter_Father
Daughter_Mother
Brother_Brother
Brother_Sister

Victim

UK1

Ref

Close

Search:

Pr (IBD = 2)	Pr (IBD = 1)	Pr (IBD = 0)	Paternal lineage	Maternal lineage
0	1	0	Yes	No
0	1	0	No	No
0	1	0	No	Yes
0	1	0	Yes	Yes
0	1	0	No	Yes
0	1	0	No	No
0	1	0	No	Yes
0.25	0.5	0.25	Yes	Yes
0.25	0.5	0.25	No	Yes

Show 10 entries Previous 1 2 3 4 5 Next

4.3 Mutation rate

Mutation rates of each autosomal STR marker need to be set to calculate the likelihood ratio (LR) for parent-child relationship. The default mutation rates considering the difference between paternal and maternal and mutational steps (-1, +1, -2, and +2) are reported by Morimoto et al [2].

To set mutation rates, select **Setting ▶ Mutation rates**.

The screenshot shows the RelSearch version 1.0.0 interface. At the top, there is a navigation bar with tabs: RelSearch ver. 1.0.0, Load, Result, Project ▾, Database ▾, Settings ▾ (which is currently active and has a dropdown menu), Example files, and Manual. Below the navigation bar, there are sections for STR, Y-ST, and mtDNA, each with fields for Victim database and Reference database, both with 'Browse...' and 'No file selected' buttons. There is also a section for Allele frequencies with similar fields. At the bottom left is a blue 'Analyze' button. A red arrow points to the 'Mutation rates' option in the open 'Settings' dropdown menu.

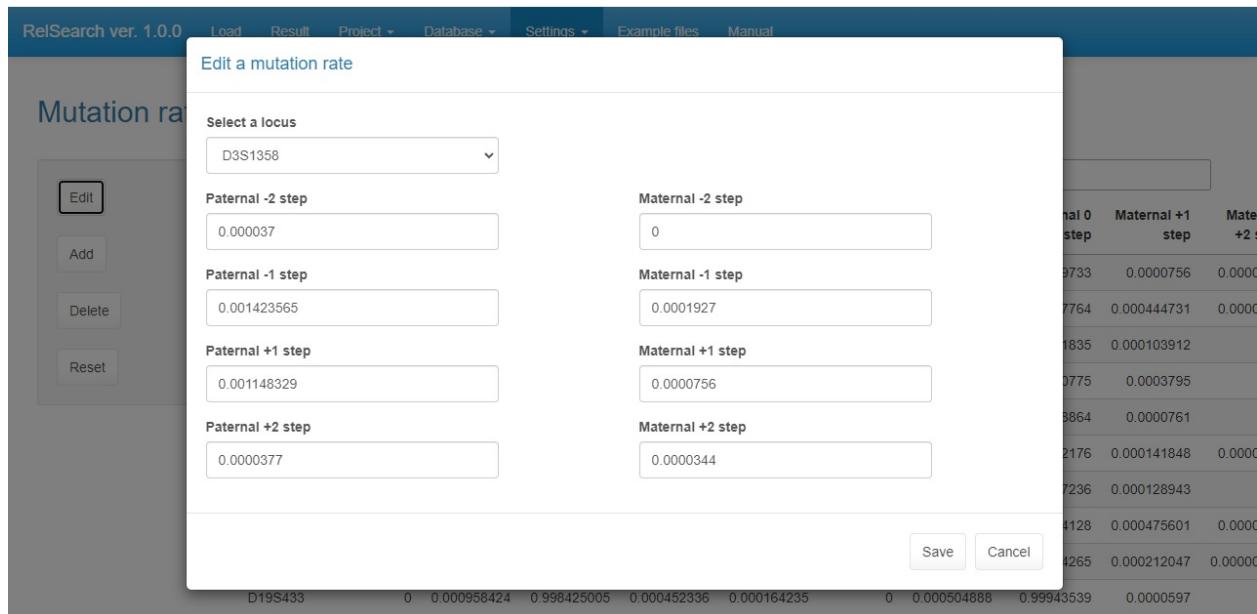
Then the setting window for mutation rates will open automatically.

Mutation rates										
<input type="button" value="Edit"/> <input type="button" value="Add"/> <input type="button" value="Delete"/> <input type="button" value="Reset"/> Search: <input type="text"/>										
Locus	Paternal -2 step	Paternal -1 step	Paternal 0 step	Paternal +1 step	Paternal +2 step	Maternal -2 step	Maternal -1 step	Maternal 0 step	Maternal +1 step	Maternal +2 step
D3S1358	0.000037	0.001423565	0.997353441	0.001148329	0.0000377	0	0.0001927	0.99969733	0.0000756	0.0000344
vWA	0.000146413	0.002718279	0.984905627	0.002200527	0.0000292	0	0.000125004	0.999377764	0.000444731	0.0000525
D16S539	0.0000841	0.00093427	0.997626583	0.001935042	0	0	0.000484253	0.999411835	0.00010912	0
CSF1PO	0	0.002042983	0.996111085	0.001845931	0	0	0.000212751	0.99940775	0.0003795	0
TPOX	0	0.000115568	0.999697045	0.000167388	0	0	0.0000751	0.999848684	0.0000761	0
D8S1179	0.0000482	0.001901255	0.997430135	0.001196466	0.0000239	0	0.000133755	0.999702176	0.000141848	0.0000222
D21S11	0.000037	0.000867972	0.998012667	0.001082339	0	0.000036	0.000107823	0.999727236	0.000128943	0
D16S51	0.00014735	0.000942663	0.997435926	0.001420767	0.0000298	0.0000213	0.000117496	0.999364128	0.000475601	0.0000215
D2S441	0.0000544	0.001166981	0.997463648	0.001250749	0.0000642	0.0000149	0.000270098	0.999494265	0.000212047	0.0000871
D19S433	0	0.000958424	0.998425005	0.000452336	0.000164235	0	0.000504888	0.99943539	0.0000597	0
TH01	0	0.0000448	0.999895495	0.0000597	0	0.000017	0.0000546	0.999920126	0.00000829	0
FGA	0.000277755	0.001497345	0.995437569	0.002707954	0.0000794	0	0.000348321	0.999350247	0.000293432	0
D22S1045	0.0000544	0.001166981	0.997463648	0.001250749	0.0000642	0.0000149	0.000270098	0.999494265	0.000212047	0.0000871
D5S818	0	0.001420549	0.9968405	0.001198	0.000540952	0	0.000258823	0.999462401	0.000278776	0
D13S317	0	0.001369203	0.997193828	0.001397392	0.0000396	0.0000333	0.0000708	0.999657082	0.000238821	0
D7S820	0	0.001302502	0.997827141	0.000870557	0	0	0.0000622	0.999813227	0.000124612	0
SE33	0.0000544	0.001166981	0.997463648	0.001250749	0.0000642	0.0000149	0.000270098	0.999494265	0.000212047	0.0000871
D10S1248	0.0000544	0.001166981	0.997463648	0.001250749	0.0000642	0.0000149	0.000270098	0.999494265	0.000212047	0.0000871
n1c16s6	0.0000544	0.001166981	0.997463648	0.001250749	0.0000642	0.0000149	0.000270098	0.999494265	0.000212047	0.0000871

4.3.1 Edit

You can edit the mutation rates from the **Edit** button.

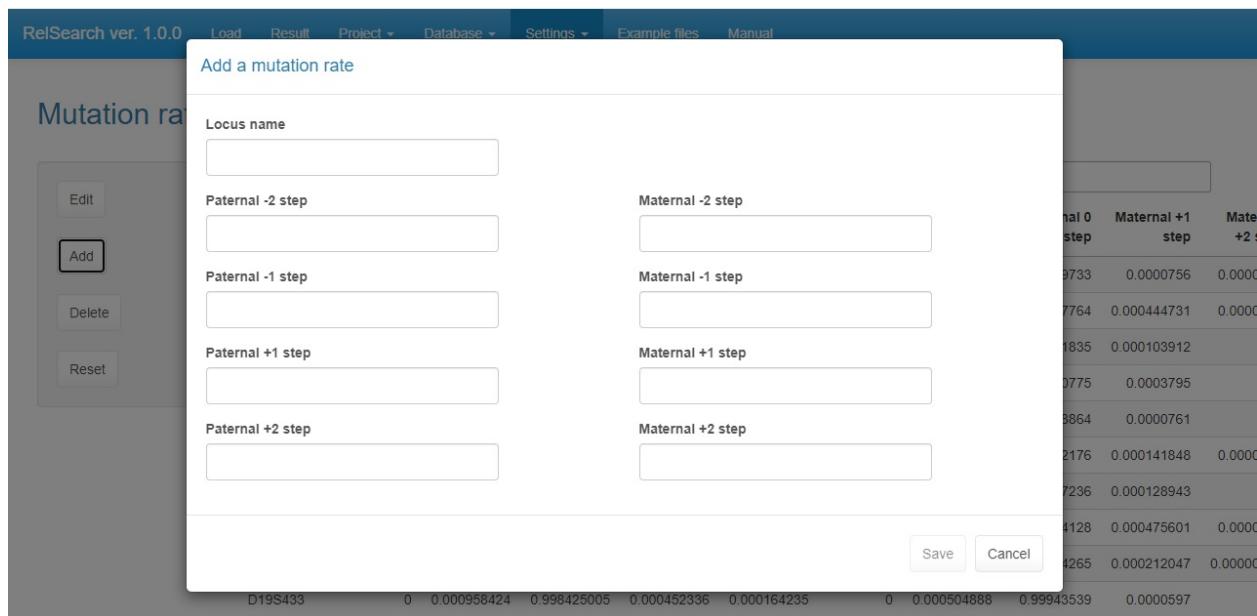
In the pop-up window, select a locus and change the mutation rates. Press the **Save** button to reflect the changes.



4.3.2 Add

You can add a locus from the **Add** button.

In the pop-up window, enter a locus name and enter the mutation rates. Press the **Save** button to add the locus.



4.3.3 Delete

You can delete a locus from the **Delete** button.

In the pop-up window, select a locus. Press the **Save** button to delete the selected locus.

The screenshot shows the RelSearch version 1.0.0 software interface. The main window has a dark blue header with tabs: Load, Result, Project, Database, Settings, Example files, and Manual. Below the header, there's a title 'Mutation rate' and a table of data. On the left, there are buttons for Edit, Add, Delete (which is highlighted with a red box), and Reset. A modal dialog box titled 'Delete a mutation rate' is overlaid on the main window. It contains a dropdown menu labeled 'Select a locus' with 'D3S1358' selected. At the bottom of the dialog are 'Save' and 'Cancel' buttons. The main table below the dialog has columns for marker names (vWA, D16S539, CSF1PO, TPOX, D8S1179, D21S11, D18S51) and various numerical values representing mutation rates across different steps.

	vWA	0.000146413	0.002718279	0.994905627	0.002200527	0.0000292	0	0.000125004	0.999377764	0.000444731	0.0000293
D16S539	0.0000841	0.00093427	0.997626583	0.001353042		0	0	0.000484253	0.999411835	0.000103912	
CSF1PO	0	0.002042983	0.996111085	0.001845931		0	0	0.000212751	0.99940775	0.0003795	
TPOX	0	0.000115566	0.999697045	0.000187388		0	0	0.0000751	0.999848864	0.0000761	
D8S1179	0.0000482	0.001301255	0.997430135	0.001196466	0.0000239	0	0.000133755	0.999702176	0.000141848	0.0000294	
D21S11	0.000037	0.000867972	0.998012667	0.001082339		0	0.000036	0.000107823	0.999727236	0.000128943	
D18S51	0.00014735	0.000942863	0.997459246	0.001420767	0.0000298	0.0000213	0.000117496	0.999364128	0.000475601	0.0000295	

4.3.4 Reset

If you want to return to default settings, press the **Reset** button. In the pop-up window, press the **Restore default** button.

The screenshot shows the RelSearch version 1.0.0 interface. The main window displays a table of mutation rates for various loci. A modal dialog box titled "Reset the mutation rates" is overlaid on the main window. The dialog contains the message "Click Restore default to remove all changes." and two buttons: "Restore default" and "Cancel".

Locus	step	step	step	step	step	-2 step	Maternal -1 step	Maternal 0 step	Maternal +1 step	Mat +2
D3S1358	0.000037	0.001423565	0.997353441	0.001148329	0.0000377	0	0.0001927	0.99969733	0.0000756	0.0000
VWA	0.000146413	0.002718279	0.994905627	0.002200527	0.0000292	0	0.000125004	0.999377764	0.000444731	0.0000
D16S539	0.0000841	0.00093427	0.997628583	0.001353042		0	0.000484253	0.999411835	0.000103912	
CSF1PO	0	0.002042983	0.996111085	0.001845931		0	0.000212751	0.99940775	0.0003795	
TPOX	0	0.000115566	0.999697045	0.000187388		0	0.0000751	0.999848864	0.0000761	
D8S1179	0.0000482	0.001301255	0.997430135	0.001196466	0.0000239	0	0.000133755	0.999702176	0.000141848	0.0000
D21S11	0.000037	0.000867972	0.998012667	0.001082339		0	0.000036	0.000107823	0.999727236	0.000128943
D18S51	0.00014735	0.000942863	0.997459246	0.001420767	0.0000298	0.0000213	0.000117496	0.999364128	0.000475601	0.0000

4.4 Data storage conditions

When handling a large number of victim and reference profiles, it may be difficult to store all the results because RelSearch may run out of memory. You can set the data storage conditions.

To set the conditions, select **Setting ▶ Data storage conditions**.

The screenshot shows the RelSearch version 1.0.0 interface. At the top, there is a navigation bar with tabs: RelSearch ver. 1.0.0, Load, Result, Project, Database, Settings (with a dropdown arrow), Example files, and Manual. The 'Settings' dropdown menu is open, showing options: Criteria, Relationships, Mutation rates, and Data storage conditions. The 'Data storage conditions' option is highlighted with a blue background and has a red arrow pointing to it from the right. Below the navigation bar, there are three main sections: STR, Y-ST, and mtDNA. Each section has fields for Victim database (Browse... and No file selected), Reference database (Browse... and No file selected), and Allele frequencies (Browse... and No file selected). At the bottom left, there is a blue 'Analyze' button.

Then the setting window for data storage will open automatically.

The screenshot shows the RelSearch software interface with a blue header bar containing the version 'RelSearch ver. 1.0.0' and several menu items: Load, Result, Project ▾, Database ▾, Settings ▾, Example files, and Manual. Below the header, the main window title is 'Data storage conditions'. A note at the top says 'Press the save button to reflect the changes.' There are two input fields: 'Minimum LR to be stored' with the value '1' and 'Maximum number of displayed data' with the value '10000'. At the bottom are two buttons: 'Save' and 'Reset'.

- Minimum LR to be stored: The minimum LR to be stored in the project data. If you want to reduce the file size of the project data, the minimum LR should be increased.
- Maximum number of displayed data: The maximum number of data displayed in the Results tab. If this number is set too high, RelSearch may run out of memory.

Press the **Save** button to reflect the changes. Press the **Reset** button if you want to return to default values.

Chapter 5

Result

This section describes the display of results on the Results tab.

5.1 Summary

When the analysis is complete, summary data is automatically displayed. You can change the data displayed using the buttons in the left sidebar. You can also download the displayed data as a .csv file using the **Download** button.

The screenshot shows the RelSearch software interface with the 'Result' tab selected. The top navigation bar includes 'RelSearch ver. 1.0.0', 'Load', 'Result', 'Project', 'Database', 'Settings', 'Example files', and 'Manual'. Below the navigation bar, there are several buttons: 'Summary' (selected), 'Selected data in detail', 'All candidates', and 'Analysis conditions'. On the left, a sidebar titled 'Display setting' contains buttons for 'Default display', 'Identified' (green), 'Inconclusive' (orange), and 'Excluded by Y-STR or mtDNA' (red). It also has a 'Minimum LR displayed' input field set to '100' and an 'Apply' button. At the bottom of the sidebar is a 'Download' button with a CSV icon. The main content area displays a table of results with the following columns: Victim, Reference, Family, Assumed relationship, LR, Estimated relationship, Estimated sex (Victim), Estimated sex (Reference), Paternal lineage, and Maternal lineage. The table shows 21 entries, with the first few rows visible:

Victim	Reference	Family	Assumed relationship	LR	Estimated relationship	Estimated sex (Victim)	Estimated sex (Reference)	Paternal lineage	Maternal lineage
V4	R4	F4	Mother_Daughter	6.33e+9	Mother_Daughter			Support	
V2	R2	F2	Father_Daughter	4.50e+8	Father_Daughter	Male		Not support	
V3	R3	F3	Mother_Son	9.01e+7	Mother_Son		Male	Support	
V1	R1	F1	Father_Son	4.12e+6	Father_Son	Male	Male	Support	Not support
V19	R19	F19	Father_Daughter	3.93e+6	Father_Daughter	Male		Not support	
V5	R5	F5	Brother_Brother	2.48e+5	Brother_Brother	Male	Male	Support	Support
V7	R7	F7	Sister_Brother	5.90e+4	Sister_Brother		Male	Support	
V22	R22	F21	Brother_Sister	1.77e+4		Male		Not support	
V21	R21	F20	Paternal-aunt_Nephew	7.95e+3	Paternal-aunt_Nephew		Male		Not support
V21	R21	F20	Paternal-aunt_Nephew	7.95e+3	Paternal-aunt_Nephew		Male		Not support

At the bottom of the table, it says 'Showing 1 to 10 of 21 entries'. To the right, there are buttons for 'Previous', page numbers (1, 2, 3, Next), and a search bar.

Default display

Default display depends on whether the input data includes autosomal STR data or not.

If the input data includes autosomal STR data, the data that satisfies the criterion of the minimum LR (see section [Setting](#)) is displayed.

If the input data is composed of only Y-STR and/or mtDNA data, the data that satisfies the criteria to support paternal/maternal lineage (see section [Setting](#)) is displayed.

If the number of data that satisfies the above conditions is more than the upper limit (i.e., the maximum number of displayed data explained in section [Data storage conditions](#)), the number of data displayed is the upper limit.

Identified

If the data supports that a victim (or a reference) has the assumed relationship with only one reference (or victim), the victim-reference pair is classified as "Identified".

Inconclusive

If the data supports that a victim (or a reference) has the assumed relationship with multiple references (or victims), the victim-reference pair is categorized as "Inconclusive".

Excluded by Y-STR or mtDNA

The victim-reference pair is excluded if the LR exceeds the minimum LR but the data does not satisfy the criteria to support paternal/maternal lineage when assuming the paternal/maternal lineage relative.

Minimum LR displayed

You can change the minimum LR displayed. After setting the value, press the **Apply** button. Please note that the amount of data stored in the project data is limited according to the data storage conditions (see [Data storage conditions](#)). Even if you set the minimum LR displayed to less than 1, you cannot check the data with $LR < 1$ as long as the minimum LR to be stored in the project data is set to 1.

5.2 Selected data in detail

The detailed data of a selected victim-reference pair is displayed in **Selected data in detail** tab.

1. Select a row in the summary table.

RelSearch ver. 1.0.0 Load Result Project Database Settings Example files Manual

Result

Summary Selected data in detail All candidates Analysis conditions

Show 10 entries Search:

Victim	Reference	Family	Assumed relationship	LR	Estimated relationship	Estimated sex (Victim)	Estimated sex (Reference)	Paternal lineage	Maternal lineage
V4	R4	F4	Mother_Daughter	6.33e+9	Mother_Daughter				Support
V2	R2	F2	Father_Daughter	4.50e+8	Father_Daughter	Male			Not support
V3	R3	F3	Mother_Son	9.01e+7	Mother_Son				Support
V1	R1	F1	Father_Son	4.12e+6	Father_Son	Male		Support	Not support
V19	R19	F19	Father_Daughter	3.93e+6	Father_Daughter	Male			Not support
V5	R5	F5	Brother_Brother	2.48e+5	Brother_Brother	Male	Male	Support	Support
V7	R7	F7	Sister_Brother	5.90e+4	Sister_Brother		Male		Support
V22	R22	F21	Brother_Sister	1.77e+4		Male			Not support
V21	R21	F20	Paternal-aunt_Nephew	7.95e+3	Paternal-aunt_Nephew		Male		Not support
V21	R21	F20	Paternal-aunt_Nephew	7.95e+3	Paternal-aunt_Nephew		Male		Not support

Showing 1 to 10 of 21 entries Previous 1 2 3 Next

2. Go to **Selected data in detail** tab.

RelSearch ver. 1.0.0 Load Result Project Database Settings Example files Manual

Result

Summary Selected data in detail All candidates Analysis conditions

Show 10 entries Search:

Victim	Reference	Family	Assumed relationship	LR	Estimated relationship	Estimated sex (Victim)	Estimated sex (Reference)	Paternal lineage	Maternal lineage
V4	R4	F4	Mother_Daughter	6.33e+9	Mother_Daughter				Support
V2	R2	F2	Father_Daughter	4.50e+8	Father_Daughter	Male			Not support
V3	R3	F3	Mother_Son	9.01e+7	Mother_Son				Support
V1	R1	F1	Father_Son	4.12e+6	Father_Son	Male		Support	Not support
V19	R19	F19	Father_Daughter	3.93e+6	Father_Daughter	Male			Not support
V5	R5	F5	Brother_Brother	2.48e+5	Brother_Brother	Male	Male	Support	Support
V7	R7	F7	Sister_Brother	5.90e+4	Sister_Brother		Male		Support
V22	R22	F21	Brother_Sister	1.77e+4		Male			Not support
V21	R21	F20	Paternal-aunt_Nephew	7.95e+3	Paternal-aunt_Nephew		Male		Not support
V21	R21	F20	Paternal-aunt_Nephew	7.95e+3	Paternal-aunt_Nephew		Male		Not support

Showing 1 to 10 of 21 entries Previous 1 2 3 Next

In **Selected data in detail** tab, you can review and download the analyzed STR, Y-STR, and mtDNA data for the selected victim-reference pair. You can check all members of the family to which the selected reference belongs.

STR tab

	STR	Y-STR	mtDNA	Likelihood (related)	Likelihood (unrelated)	LR
Selected victim						
V5						
Selected reference	D3S1358	15, 17	15, 17	0.0704	0.0257	2.74
R5	vWA	16, 18	14, 19	0.000584	0.00234	0.250
Estimated relationship	D16S539	9, 12	9, 13	0.00369	0.00615	0.599
Brother_Brother	CSF1PO	10, 10	12, 12	0.00206	0.00823	0.250
Paternal lineage	TPOX	11, 11	10, 11	0.00290	0.00304	0.951
Support	D8S1179	13, 13	11, 13	0.00330	0.00243	1.36
Maternal lineage	D21S11	29, 30	30, 31.2	0.00473	0.00766	0.617
Support	D18S51	11, 19	11, 19	0.0000951	1.33e-7	714
All family members	D2S441	11, 14	10, 11	0.00767	0.0125	0.614
R5	D19S433	14, 15.2	13, 15.2	0.00700	0.00521	1.34
	TH01	6, 9	6, 6	0.0118	0.00851	1.38
	FGA	22, 24	22, 24	0.0219	0.00382	5.72
	D22S1045	11, 15	11, 15	0.0501	0.0151	3.32
	DSS818	10, 11	12, 12	0.00156	0.00624	0.250
	D13S317	8, 9	8, 9	0.0249	0.00466	5.35
	D7S820	10, 11	10, 11	0.0619	0.0213	2.90
	SE33	19, 26.2	19, 26.2	0.00359	0.000150	23.9
	D10S1248	14, 15	13, 15	0.0138	0.0171	0.806
	D1S1656	14, 15	12, 14	0.000475	0.000275	1.73
	D12S391	18, 19	18, 24	0.00118	0.00159	0.744
	D2S1338	21, 23	23, 23	0.000170	0.0000837	2.04
	Total			1.06e-50	4.27e-56	2.48e+5

Y-STR tab

Summary Selected data in detail All candidates Analysis conditions

STR Y-STR mtDNA

Selected victim	V5	Selected reference	R5	Estimated relationship	Brother_Brother	Paternal lineage	Support	Maternal lineage	Support	All family members	R5
Locus	Victim profile	Reference profile	Ignored locus	Mismatched locus	Mutational step						
DYS576	20	20	0	0	0						
DYS389I	13	13	0	0	0						
DYS635	21	21	0	0	0						
DYS389II	29	29	0	0	0						
DYS627	19	20	0	1	1						
DYS460	10	10	0	0	0						
DYS458	19	19	0	0	0						
DYS19	15	15	0	0	0						
GATA_H4	12	12	0	0	0						
DYS448	18	18	0	0	0						
DYS391	8	8	0	0	0						
DYS456	15	15	0	0	0						
DYS390	22	22	0	0	0						
DYS438	13	13	0	0	0						
DYS392	13	13	0	0	0						
DYS518	42	42	0	0	0						
DYS570	17	17	0	0	0						
DYS437	13	13	0	0	0						
DYS385	10,17	10,17	0	0	0						
DYS449	30	30	0	0	0						
DYS393	14	14	0	0	0						
DYS439	12	12	0	0	0						
DYS481	21	21	0	0	0						
DYF387S1	37	37	0	0	0						
DYS533	11	11	0	0	0						
Total			0	1	1						

[Download \(STR\)](#)
 [Download \(Y-STR\)](#)
 [Download \(mtDNA\)](#)

mtDNA tab

Summary Selected data in detail All candidates Analysis conditions

STR Y-STR mtDNA

Number of mismatches	Shared range	Shared length (bp)	
0	73-340 16024-16365	610	
Victim profile	Reference profile	Out of shared range	Mismatch
73G	73G		
152C	152C		
263G	263G		
16071T	16071T		
16093C	16093C		
16109G	16109G		
16129A	16129A		
16223T	16223T		
16249C	16249C		
16362C	16362C		

[Download \(STR\)](#)

[Download \(Y-STR\)](#)

[Download \(mtDNA\)](#)

5.3 All candidates

The data may support that a victim (or a reference) is related to not only one reference (or victim), but to multiple references (or victims). In this case, the **All Candidates** tab allows you to review all candidate relatives who are estimated to be related to the targeted victim or reference.

1. Select a row in the summary table.

This screenshot shows the RelSearch software interface. The top navigation bar includes 'RelSearch ver. 1.0.0', 'Load', 'Result', 'Project', 'Database', 'Settings', 'Example files', and 'Manual'. The 'Result' tab is selected. Below it, there are tabs for 'Summary', 'Selected data in detail', 'All candidates', and 'Analysis conditions'. The 'Summary' tab is currently active. On the left, a 'Display setting' panel has 'Default display' selected. It also includes buttons for 'Identified' (green), 'Inconclusive' (orange), and 'Excluded by Y-STR or mtDNA' (red). A 'Minimum LR displayed' input field is set to 100, and an 'Apply' button is present. A 'Download' button is at the bottom. The main area shows a table titled 'Show 10 entries' with columns: Victim, Reference, Family, Assumed relationship, LR, Estimated relationship, Estimated sex (Victim), Estimated sex (Reference), Paternal lineage, and Maternal lineage. The table lists 21 entries. The 19th entry (V19, R19, F19) is highlighted in blue and has a red arrow pointing to its 'Not support' status in the 'Estimated sex (Reference)' column. The table footer indicates 'Showing 1 to 10 of 21 entries' and includes page navigation buttons for 'Previous', '1', '2', '3', and 'Next'.

2. Go to **All candidates** tab.

This screenshot shows the same RelSearch software interface as the previous one, but the 'All candidates' tab is now selected. The top navigation bar and tabs are identical. The 'Display setting' panel on the left remains the same. The main table in the center also shows the same 21 entries. The 19th entry (V19, R19, F19) is highlighted in blue and has a red arrow pointing to its 'Not support' status in the 'Estimated sex (Reference)' column. The table footer indicates 'Showing 1 to 10 of 21 entries' and includes page navigation buttons for 'Previous', '1', '2', '3', and 'Next'.

Then, all candidate victim-reference pairs that are related to the selected victim and reference are displayed. You can download the data displayed.

The screenshot shows the RelSearch software interface. At the top, there is a navigation bar with links: RelSearch ver. 1.0.0, Load, Result (which is currently selected), Project, Database, Settings, Example files, and Manual. Below the navigation bar, the word "Result" is displayed in bold. Under "Result", there are several tabs: Summary, Selected data in detail (which is selected), All candidates, and Analysis conditions. On the left, there is a sidebar with sections for "Selected victim" (V19) and "Selected reference" (R19). The main area displays a table of results with the following columns: Victim, Reference, Family, Assumed relationship, LR, Estimated relationship, Estimated sex (Victim), Estimated sex (Reference), Paternal lineage, and Maternal lineage. The table shows two entries:

Victim	Reference	Family	Assumed relationship	LR	Estimated relationship	Estimated sex (Victim)	Estimated sex (Reference)	Paternal lineage	Maternal lineage
V19	R19	F19	Father_Daughter	3.93e+6	Father_Daughter	Male			Not support
V19	R20	F19	Paternal_uncle_Niece	1.48	Paternal_uncle_Niece	Male			Not support

At the bottom left of the main area, there is a blue "Download" button with a downward arrow icon. At the bottom right, there are buttons for "Previous", "1" (selected), and "Next". A search bar is located at the top right of the main area.

5.4 Analysis conditions

You can review the following analysis conditions in **Analysis conditions** tab.

- **Database:** Input .csv file names of each database are displayed.
- **Allele probability:** Unobserved alleles in the population database are displayed, if any. You can download the allele probabilities that are estimated based on Dirichlet distribution (see section [Computational principle](#)).
- **Criteria:** The user-defined criteria to determine whether each victim-reference pair has an assumed relationship or not (see section [Setting](#)) is displayed.
- **Assumed relationship:** Information on the user-defined relationships (see section [Setting](#)) is displayed.
- **Mutation rate:** The user-defined mutation rates (see section [Setting](#)) are displayed.
- **Data storage conditions:** The user-defined conditions for data storage (see section [Setting](#)) is displayed.

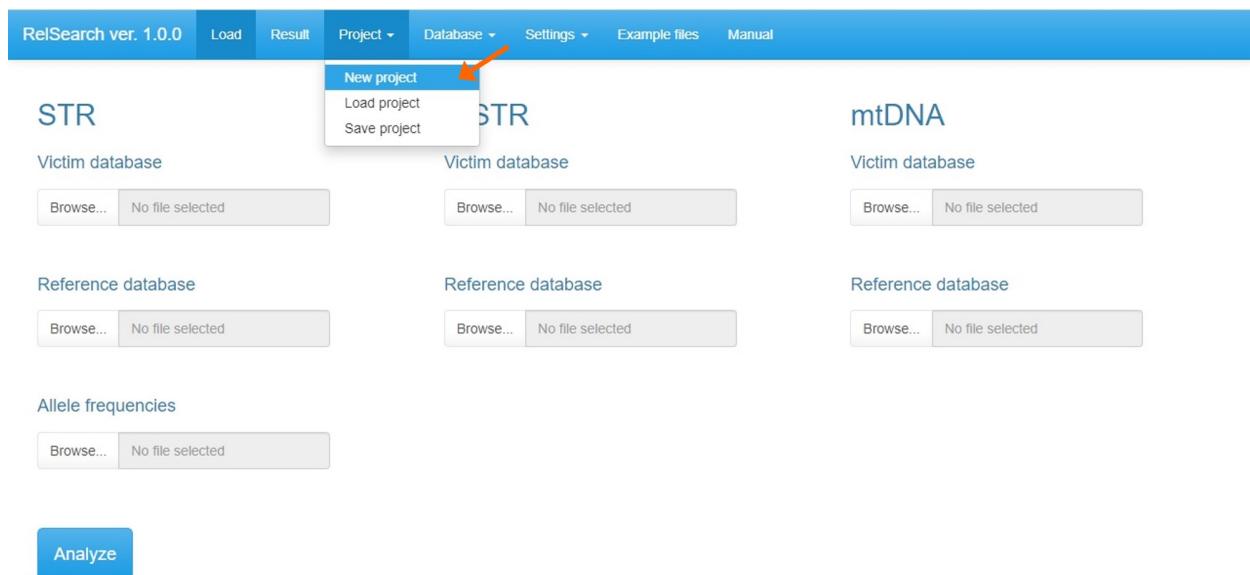
Chapter 6

Other functions

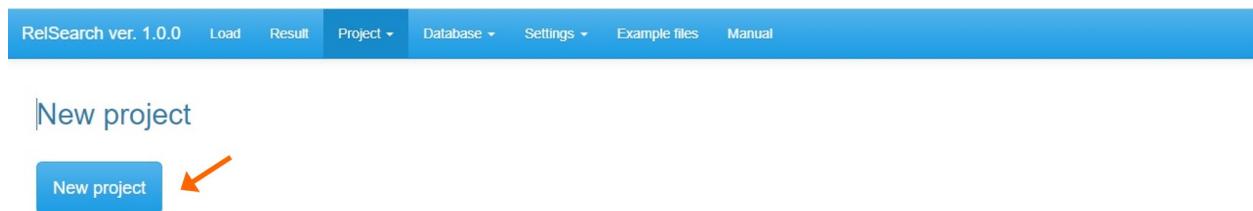
6.1 Project

6.1.1 New project

To start a new project, go to **Project ▶ New project**.



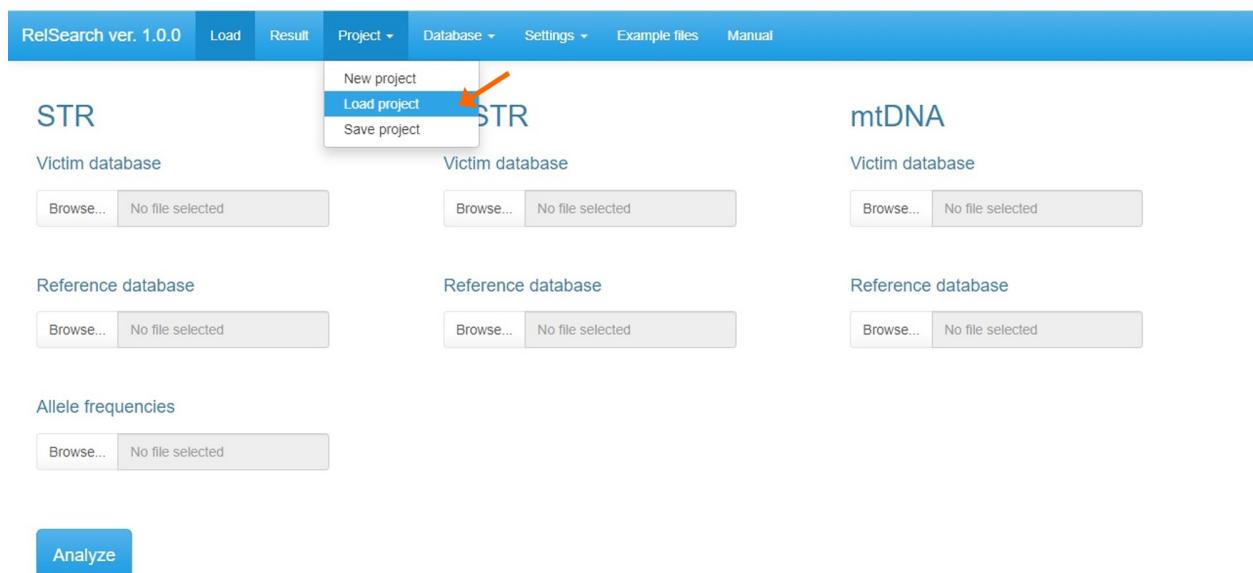
Press the **New project** button.



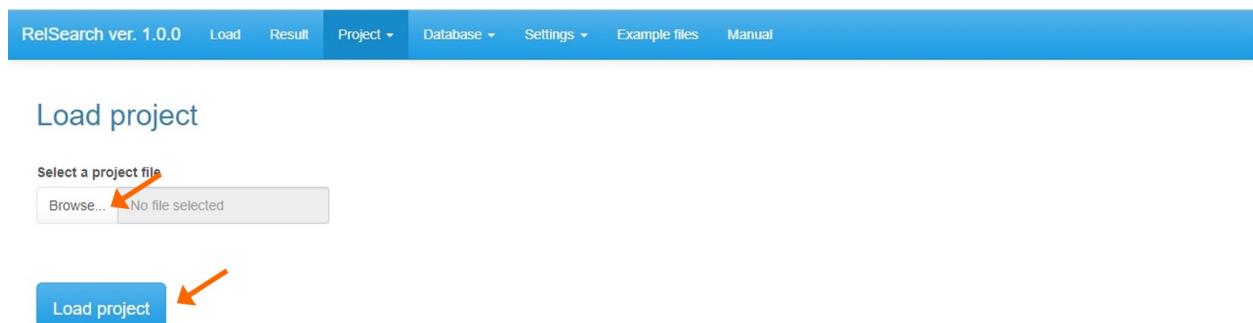
Important: The current project should be saved before starting a new project because the current project will be deleted.

6.1.2 Load project

To load a previous project, go to **Project ▶ Load project**.



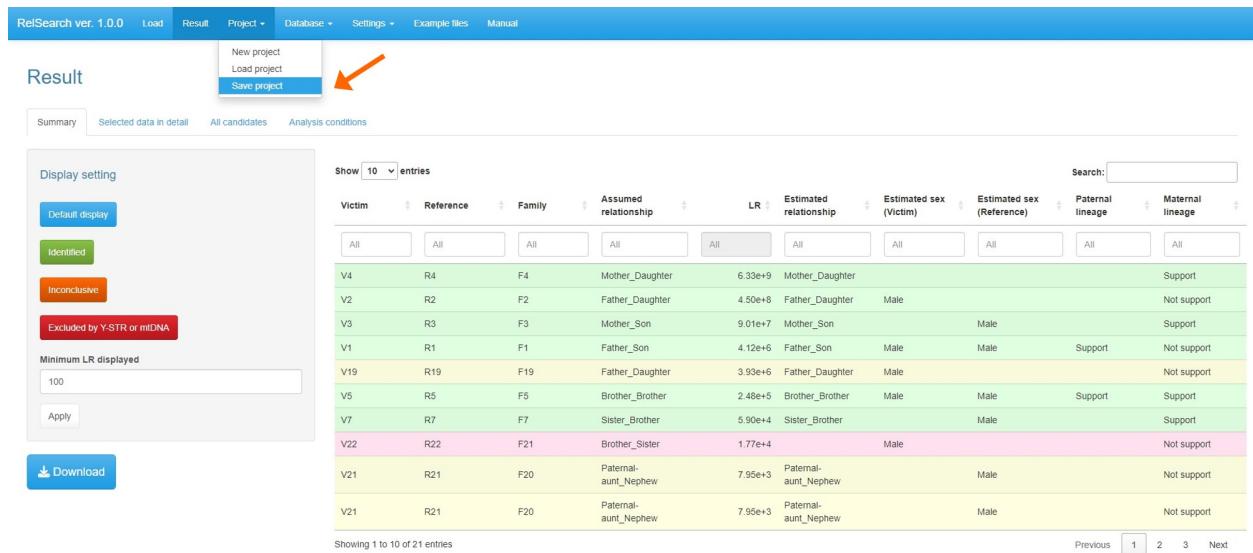
Select a project file from the **Browse...** button and press the **Load project** button.



Important: The current project should be saved before loading another project because the current project will be deleted.

6.1.3 Save project

To save the current project, go to **Project ▶ Save project**.



The screenshot shows the RelSearch software interface. At the top, there is a blue header bar with the text "RelSearch ver. 1.0.0" and several menu items: Load, Result, Project (with a dropdown arrow), Database (with a dropdown arrow), Settings (with a dropdown arrow), Example files, and Manual. Below the header, the word "Result" is displayed. In the top right corner of the main area, there is a small dropdown menu with three options: "New project", "Load project", and "Save project". A red arrow points to the "Save project" option. The main content area has tabs at the top: Summary, Selected data in detail, All candidates, and Analysis conditions. On the left, there is a sidebar titled "Display setting" with buttons for "Default display", "Identified", "Inconclusive", and "Excluded by Y-STR or mtDNA". It also includes a "Minimum LR displayed" input field set to "100" and an "Apply" button. Below this is a "Download" button. The main table area shows 10 entries out of 21 total. The columns include Victim, Reference, Family, Assumed relationship, LR, Estimated relationship, Estimated sex (Victim), Estimated sex (Reference), Paternal lineage, and Maternal lineage. The table rows contain various family relationships like Mother_Daughter, Father_Son, etc., with corresponding LR values and lineage information. At the bottom of the table, it says "Showing 1 to 10 of 21 entries" and has navigation buttons for Previous, Next, and page numbers 1, 2, 3.

Enter the project name and press the **Save as** button.



The screenshot shows the "Save project" dialog box. At the top, there is a blue header bar with the text "RelSearch ver. 1.0.0" and several menu items: Load, Result, Project (with a dropdown arrow), Database (with a dropdown arrow), Settings (with a dropdown arrow), Example files, and Manual. Below the header, the title "Save project" is displayed. The main area has a label "Enter the project name" followed by an input field. A red arrow points to this input field. At the bottom is a blue "Save as" button with a download icon, and another red arrow points to it.

6.2 View database

Each loaded database can be reviewed in **Database** tab.

The screenshot shows the RelSearch version 1.0.0 software interface. At the top, there is a navigation bar with tabs: RelSearch ver. 1.0.0, Load, Result, Project, Database (selected), Settings, Example files, and Manual. Below the navigation bar, the interface is divided into two main sections: STR and mtDNA.

STR Section:

- Victim database:** A file selection box labeled "Browse..." with "No file selected".
- Reference database:** A file selection box labeled "Browse..." with "No file selected".
- Allele frequencies:** A file selection box labeled "Browse..." with "No file selected".
- Database dropdown menu:** A dropdown menu containing the following items:
 - STR : Victim
 - STR : Reference
 - STR : Allele frequencies
 - Y-STR : Victim
 - Y-STR : Reference
 - mtDNA : Victim
 - mtDNA : Reference

mtDNA Section:

- Victim database:** A file selection box labeled "Browse..." with "No file selected".
- Reference database:** A file selection box labeled "Browse..." with "No file selected".

Analyze button: A blue button labeled "Analyze" located at the bottom left of the STR section.

6.3 Example files

Example files for each database can be downloaded from the **Example files** tab.

The screenshot shows the RelSearch software interface. At the top, there is a blue header bar with the following menu items: 'RelSearch ver. 1.0.0', 'Load', 'Result', 'Project ▾', 'Database ▾', 'Settings ▾', 'Example files' (which is highlighted in blue), and 'Manual'. Below the header, the main content area has a title 'Example files' in blue. Underneath this title, there is a vertical list of download links, each enclosed in a light gray box with a small download icon: 'STR victim database', 'STR reference database', 'Allele frequencies', 'Y-STR victim database', 'Y-STR reference database', 'mtDNA victim database', and 'mtDNA reference database'.

Chapter 7

Computation time and limits

When handling a large number of victim and reference profiles, the computational load of RelSearch will be high. This section provides approximate computation times and limits as a guide.

The following 6 databases have been analyzed to estimate computation time and limits.

ID	No. of victims (V)	No. of references (R)	No. of V-R pairs	Actual V-R relationship (n = 1,000 in each)
1	1,000	1,000	1 million	BB
2	2,000	2,000	4 million	BB, FS
3	3,000	3,000	9 million	BB, FS, MD
4	4,000	4,000	16 million	BB, FS, MD, SS
5	5,000	5,000	25 million	BB, FS, MD, SS, UN
6	6,000	6,000	36 million	BB, FS, MD, SS, UN, AN

BB: brothers (marker type: STR, Y-STR, mtDNA)

FS: father-son (marker type: STR, Y-STR)

MD: mother-daughter (marker type: STR, mtDNA)

SS: sisters (marker type: STR, mtDNA)

UN: uncle-nephew (marker type: STR, Y-STR)

AN: aunt-niece (marker type: STR, mtDNA)

The targeted autosomal STR loci are 21 loci typed by the GlobalFiler™ PCR Amplification Kit (Thermo Fisher Scientific, Waltham, MA). The targeted Y-STR loci are 27 loci typed by Yfiler™ Plus PCR Amplification Kit (Thermo Fisher Scientific). The targeted mtDNA regions were the hypervariable regions (HVR) I (16024-16365) and II (73-340).

Computer specifications are Windows 10 64 bit, Intel Core i9-9900K CPU, 3.6 GHz, 32 GB RAM. Data storage conditions are as follows:

- Minimum LR to be stored: 1
- Maximum number of displayed data: 10,000

The following table shows approximate computation times and warnings related to computation limits.

ID	Computational time	Warning message in the R console
1	4 min	
2	12 min	
3	26 min	
4	53 min	
5	94 min	It seems your data is too big.
6	157 min	It seems your data is too big.

All 6 databases could be analyzed, but a warning message related to computation limits was displayed in the R console when analyzing database IDs 5 and 6 (i.e., performing more than 25 million comparisons between victim and reference profiles). In addition, the number of victim-reference pairs that meet the criteria for kinship estimation has reached the upper limit of the data displayed (i.e., 10,000) when analyzing database IDs 5 and 6. These results suggest that the upper limit of computation is less than 25 million comparisons. Please note that the limit is based only on the computer specifications and databases used in this study.

Chapter 8

Computational principle

8.1 STR

8.1.1 Likelihood ratio

The likelihood ratio (LR) is calculated for each victim-reference pair by assuming the following two hypotheses:

- H_1 : The victim and the reference are a certain relationship,
- H_2 : The victim and the reference are unrelated.

The equation of the LR is as follows:

$$LR = \frac{Pr(G_v, G_r | H_1)}{Pr(G_v, G_r | H_2)} \quad (1)$$

where G_v and G_r denote the genotypes of the victim and the reference, respectively.

In RelSearch, users can set the minimum threshold of the LR to estimate that the victim and reference have the relationship assumed in H_1 . The default threshold is 100, which is determined based on the verbal scale "Moderate Support" written in the Scientific Working Group on DNA Analysis Methods (SWGDDAM) guideline [3].

If full profiles are obtained for a victim-reference pair under the assumption of the relationship other than the parent-child (i.e., without considering drop-out and mutations in calculation), the numerator and the denominator of the LR are calculated using the probabilities that two, one and zero alleles are identity by descent (IBD).

$$\begin{aligned} Pr(G_v, G_r | H) &= Pr(G_v, G_r | IBD = 2)Pr(IBD = 2 | H) \\ &\quad + Pr(G_v, G_r | IBD = 1)Pr(IBD = 1 | H) \\ &\quad + Pr(G_v, G_r | IBD = 0)Pr(IBD = 0 | H) \end{aligned} \quad (2)$$

The specific method for these calculations is based on the method of Wenk et al [4].

8.1.2 Allele probabilities

The allele probabilities are assumed to be based on Dirichlet distributions in each locus [2]. The probability of allele x (p_x) can be estimated using the following formula:

$$p_x = \frac{\alpha_x + 1}{\sum_{x=1}^X (\alpha_x + 1)} \quad (3)$$

where α_x is the number of observations of the x th allele ($x = 1, 2, \dots, X$) and X is the number of observed allele types.

If some alleles of victims or references are unobserved in the population database, these alleles are added to the observed allele types as zero observation (i.e., $\alpha_x = 0$), and p_x of all alleles are re-estimated [2].

8.1.3 Allelic drop-out

When only one allele is observed and another allele may have dropped out in a locus, RelSearch assumes that the genotype is both homozygous (i.e., no drop-out allele) and heterozygous (i.e., one drop-out allele). The probability of observing the profile is the same in all candidate genotypes. The method corresponds to the "Method 1" proposed by Dørum et al [5].

Here, G_v^* and G_r^* denote the observed profiles of the victim and the reference, respectively. G_{v_i} and G_{r_j} denote the i th candidate genotype of the victim and the j th candidate genotype of the reference, respectively.

Suppose that G_v^* is 15/- (i.e., partial profile) and G_r^* is 16/17. In this case, RelSearch considers the following candidate genotypes of the victim:

G_{v_1} : 15/15,

G_{v_2} : 15/16,

G_{v_3} : 15/17, and

G_{v_4} : 15/Q.

Q denotes any undetected alleles other than 15, 16, and 17.

The candidate genotype of the reference is only 16, 17 because two heterozygote alleles are detected.

In this case, the likelihood function considering allelic drop-out is as follows:

$$\begin{aligned}
& Pr(G_v^* : 15/-, G_r^* : 16/17|H) \\
& = Pr(G_v^* : 15/-, G_r^* : 16/17|G_{v_i} : 15/15, G_r : 16/17)Pr(G_{v_i} : 15/15, G_r : 16/17|H) \\
& + Pr(G_v^* : 15/-, G_r^* : 16/17|G_{v_i} : 15/16, G_r : 16/17)Pr(G_{v_i} : 15/16, G_r : 16/17|H) \\
& + Pr(G_v^* : 15/-, G_r^* : 16/17|G_{v_i} : 15/17, G_r : 16/17)Pr(G_{v_i} : 15/17, G_r : 16/17|H) \\
& + Pr(G_v^* : 15/-, G_r^* : 16/17|G_{v_i} : 15/Q, G_r : 16/17)Pr(G_{v_i} : 15/Q, G_r : 16/17|H)
\end{aligned} \tag{4}$$

This equation is generalized as follows:

$$Pr(G_v^*, G_r^*|H) = \sum_i \sum_j Pr(G_v^*, G_r^*|G_{v_i}, G_{r_j})Pr(G_{v_i}, G_{r_j}|H) \tag{5}$$

$Pr(G_v^*, G_r^*|G_{v_i}, G_{r_j})$ is assumed to be the same irrespective of the candidate genotypes. Therefore, the likelihood function can be rewritten as follows:

$$Pr(G_v^*, G_r^*|H) = \sum_i \sum_j Pr(G_{v_i}, G_{r_j}|H) \tag{6}$$

$Pr(G_{v_i}, G_{r_j}|H)$ values are calculated according to equation (2) under the assumption of the relationship other than the parent-child. When assuming the parent-child relationship, mutational events are considered for the calculation as explained in the next session.

8.1.4 Mutation

When assuming the parent-child relationship, mutational events are considered for the calculation of $Pr(G_{v_i}, G_{r_j}|H)$ in equation (6). Suppose that a victim-reference pair is assumed to be parent-child, and G_{v_i} is a/b and G_{r_j} is c/d. RelSearch assumes all inheritance patterns from the parent to the child including mutational events. The LR is calculated according to [6,7].

$$LR = \frac{(\mu_{a \rightarrow c} + \mu_{b \rightarrow c})p_d + (\mu_{a \rightarrow d} + \mu_{b \rightarrow d})p_c}{4p_c p_d} \tag{7}$$

where p_a , p_b , p_c , and p_d denote the probabilities of the allele a , b , c , and d , respectively. $\mu_{a \rightarrow c}$ denotes the probability that the parent allele a is inherited as the allele c to the child. $\mu_{a \rightarrow c}$, $\mu_{b \rightarrow c}$, $\mu_{a \rightarrow d}$, and $\mu_{b \rightarrow d}$ depend on the sex of the parent (i.e., paternal or maternal), and the mutational steps are assumed to be -2, -1, +1, and +2 steps. The mutation rates reported by Morimoto et al [2] are used as the default values.

8.2 Y-STR

RelSearch analyzes the number of mismatched loci and the total mutational steps between victim and reference Y-STR profiles. The following table shows some examples of the analysis results in one locus.

Victim profile	Reference profile	Mismatch	Mutational steps	Ignore due to allelic drop-out
15	15		0	
15	16	Yes	1	
15	17	Yes	2	
-	15			Yes
15	15, 17			Yes
15	16, 17	Yes	1	
15, 16	15, 17	Yes	1	
15, 16	15, 18	Yes	2	
15, 17	16, 18	Yes	Unable to calculate	

RelSearch ignores loci where the victim and reference profile match when considering the allelic drop-out. Suppose that the victim profile is 15, and the reference profile is 15, 17 in a duplicated marker (e.g., DYS385). If the allele 17 is assumed to be dropped out in the victim profile, the assumed victim profile is the same as the reference profile.

If multiple mutational events are assumed in a duplicated marker, RelSearch does not calculate the mutational steps for the marker due to the difficulty of the estimation of the actual mutational process (i.e., "Unable to calculate" in the above table). Especially in DYF387S1, three alleles may be observed and the mutational process related to these alleles is too complex.

After analyses of all victim-reference profiles, RelSearch investigates whether each victim-reference profile supports paternal lineage or not based on the following criteria.

- Maximum number of mismatched loci: The upper limit of the number of mismatched loci between victim and reference profiles for which paternal lineage is not negated.
- Maximum total mutational steps: The upper limit of total mutational steps between victim and reference profiles for which paternal lineage is not negated.

The default criteria for the maximum number of mismatched loci is 4 and for the maximum total mutational steps is 5, which is determined based on a previous study [8].

8.3 mtDNA

RelSearch investigates the number of mismatched nucleotides between the victim and the reference sequences. There is one criterion to support maternal lineage as follow:

- Maximum number of inconsistency: The upper limit of inconsistency between victim and reference profiles for which maternal lineage is not negated.

Information on the ranges of the mtDNA sequences in each profile is needed because RelSearch targets the ranges read in both victim and reference profiles. The mtDNA profiles are expressed as the differences to a reference sequence such as the revised Cambridge Reference Sequence [1] (e.g., 73G, 315.1C, and so on). RelSearch only recognizes differences in strings of each mtDNA profile; therefore, the rule of the notation for mtDNA profiles should be aligned in each profile by software users.

The default maximum number of inconsistency is 1, which is determined according to the SWGDAM guideline for mtDNA analysis interpretation [9].

Reference

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