Help

Chapter 1

Create Design

1.1 Design tools interface

After accessing GenoDesigner, you will encounter the interface which includes the navigations, “Design Tool” and “Biological Part Management”, on the left sidebar, allowing you to switch between interfaces by clicking on them. In the center of the “Design Tool” interface, you will find the design list, which enables you to search for designs. Positioned on the right is the "New Design" button (Fig. 1.1).

Fig. 1.1: Main interface of GenoDesigner.

1.2 New Design

Navigate to the Design Tool in the left sidebar. Click "New Design", input the design name, and select “Blank Design” or “Choose a Template”. By default, the creation method is set to generate a new blank design. Upon providing the required information, click "OK" to proceed to the design page (Fig. 1.2). Creating a design from a template requires previously saved templates (See 5.3: Save design as a template). Note: When creating from templates, you can search for existing templates using fuzzy queries.

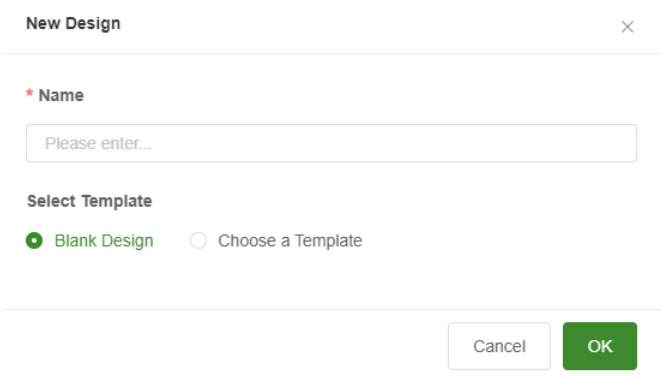

Fig. 1.2: Create new design.

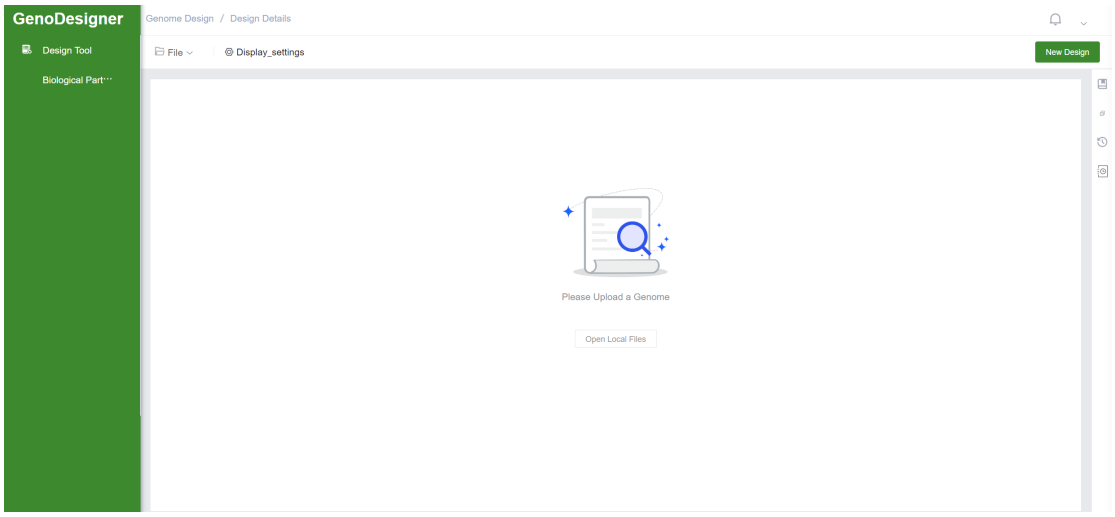

Fig. 1.3: Blank design interface.

1.3. Import sequences

Once you create a blank design, genome sequences must be imported as basis for modification. By clicking on “File”, 'Open Files', a file upload dialog will appear, allowing you to upload sequence and annotation files in FASTA, Genbank, GTF, GFF, and GFF3 format (*.fa, *.gb, *gbk, *gtf, *gff, *gff3) at the same time with support for multiple selections. The annotation files can be imported alone into current sequences by clicking “File” and “Open Annotation Files” but cannot be imported alone into a blank design. Once the files are opened, you can browse and edit the sequences (Fig. 1.4). If you want to check the difference between multiple sequences, you can select multiple Genbank files while importing sequences, ensuring that the number and length of chromosomes in each file match. After opening the files, the system will display the chromosome views of all files in a multi-track display, but editing is not allowed at this stage (Fig. 1.5). Users can browse and select annotation information from one of the files, and once selected, it cannot be changed (Fig. 1.6). Editing and modifications can be made based on the selected genome file.

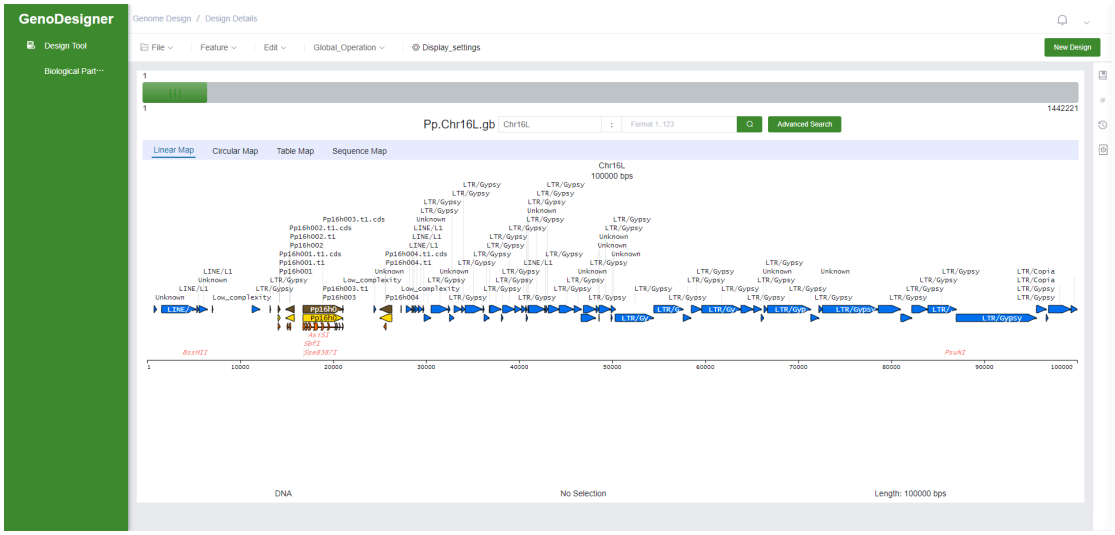

Fig. 1.4: The interface after importing the sequence.

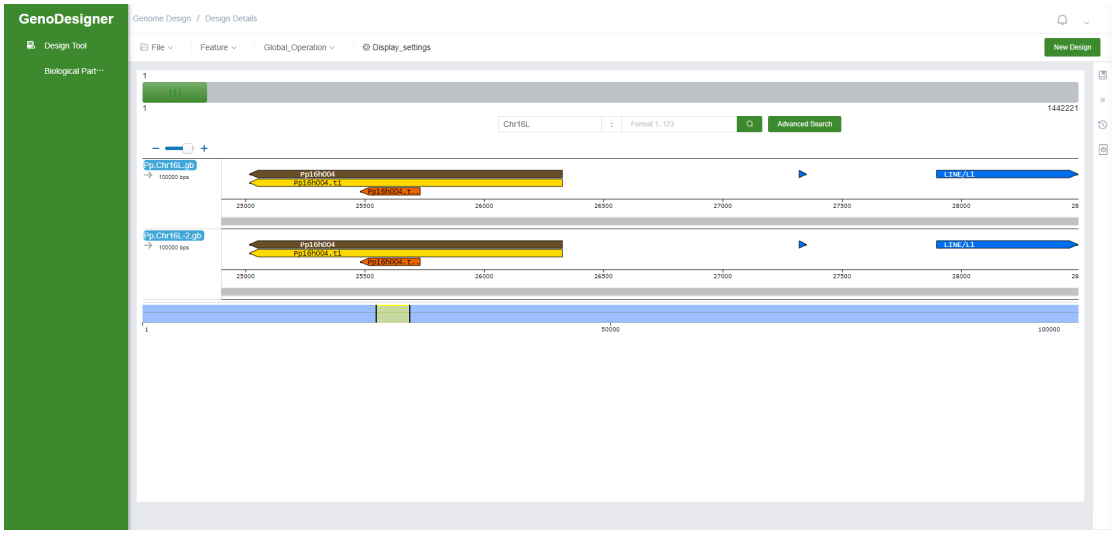

Fig. 1.5: Display of multiple sequences.

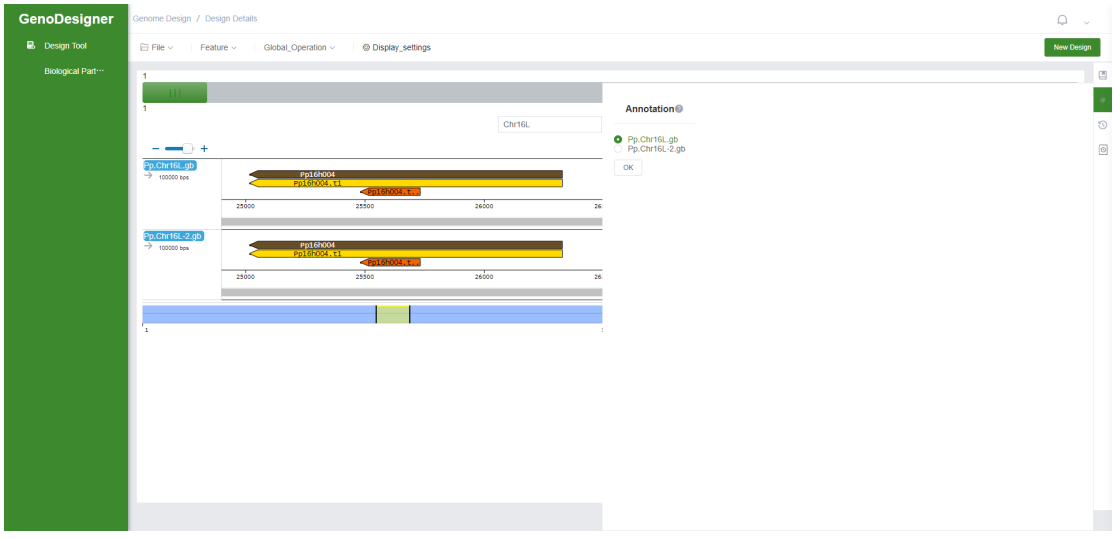

Fig. 1.6: select one of the sequences to edit.

If mistakes were made while creating or editing a new design, you can reopen the upload file and confirm overwriting the current file by clicking "File" and “Open Sequence Files” (Fig. 1.7). You can also import additional annotation files for the current genome by clicking “File” and “Open Annotation Files”.

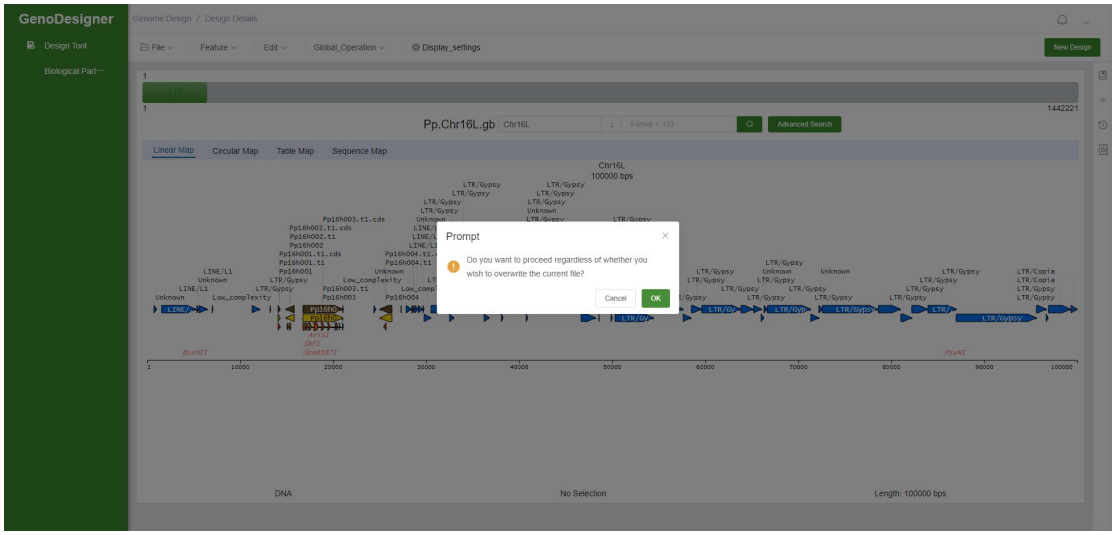

Fig. 1.7: Overwrite current sequences.

Chapter 2

Genome Visualization and Feature Operations

2.1 Chromosome View

After importing genome files, a chromosome list will appear, allowing for selection. Upon selecting, clicking "OK" will display the features of the selected chromosome (Fig. 2.1). GenoDesigner supports the browsing switch among four types of views: linear map (Fig. 2.2), circular map (Fig. 2.3), table map (feature list) (Fig. 2.4), and sequence map (Fig. 2.5). Furthermore, It also supports dragging the four types of graphs left and right to form a double-view for inspection (Fig. 2.6).

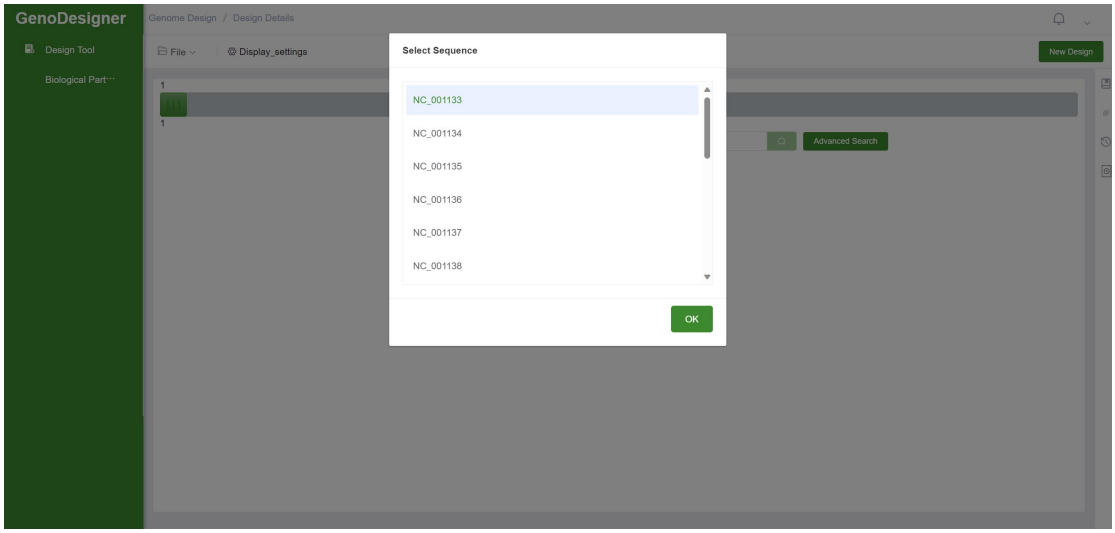

Fig. 2.1: Select sequence to display.

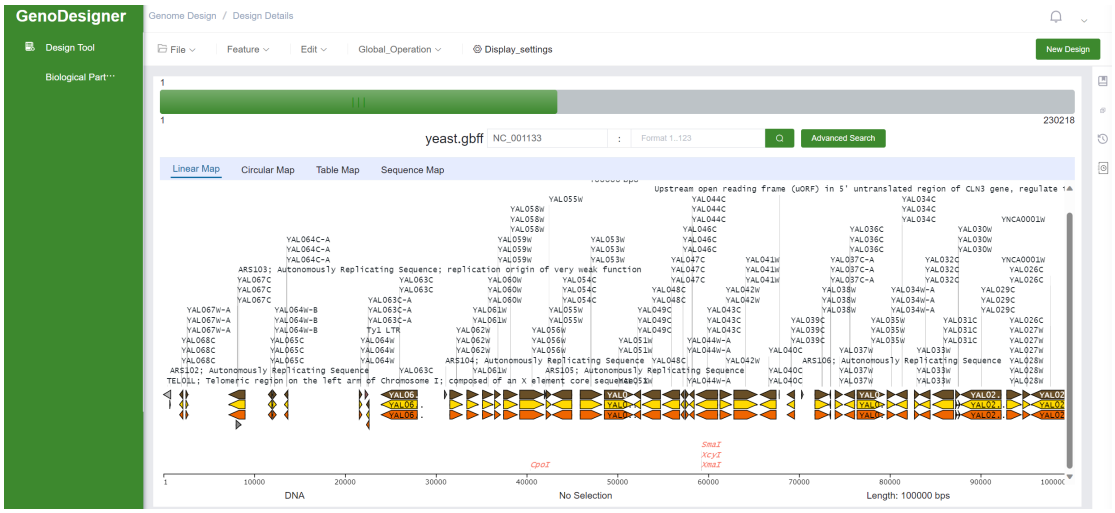

Fig. 2.2: Linear map.

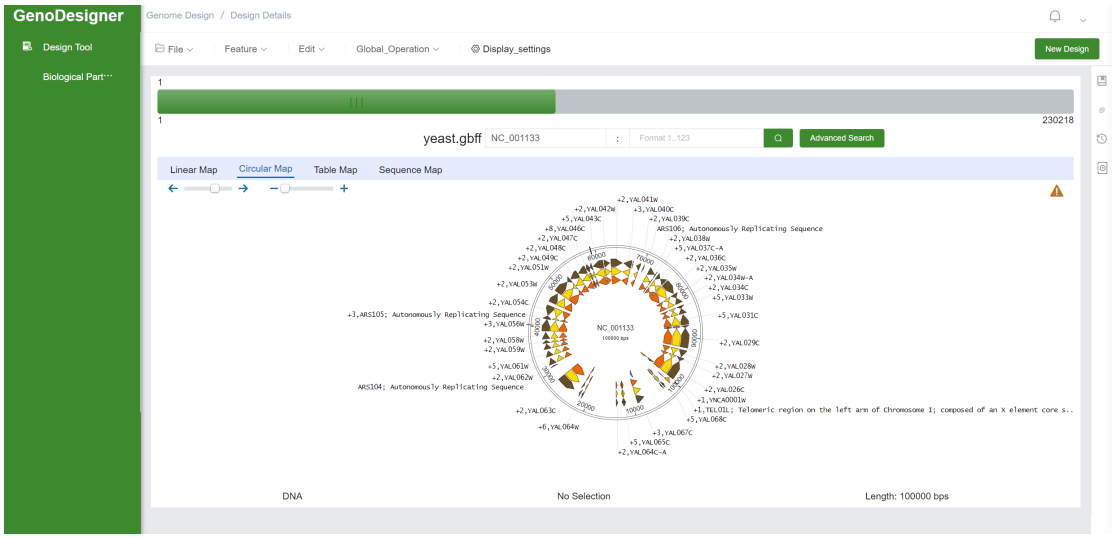

Fig. 2.3: Circular map.

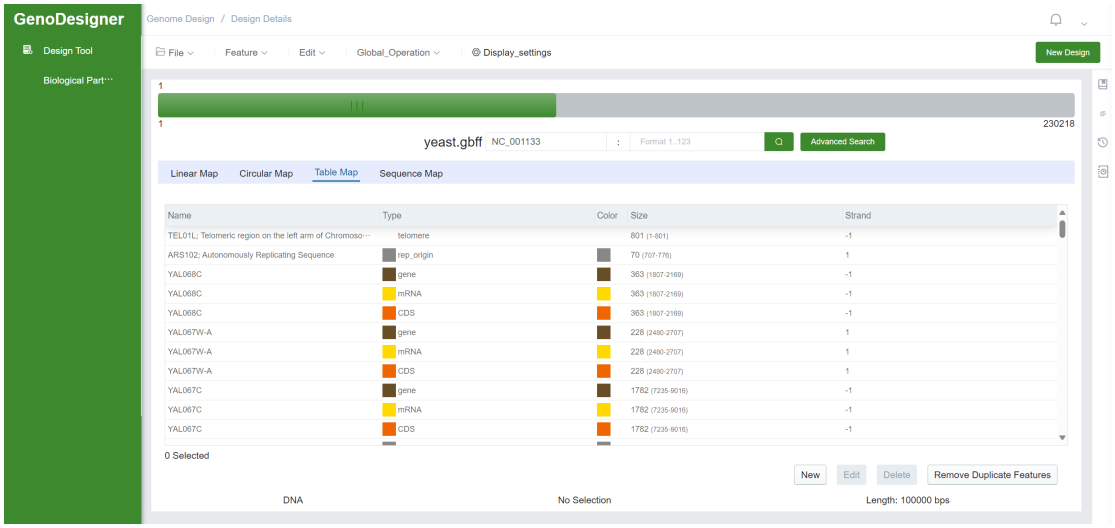

Fig. 2.4: Table map.

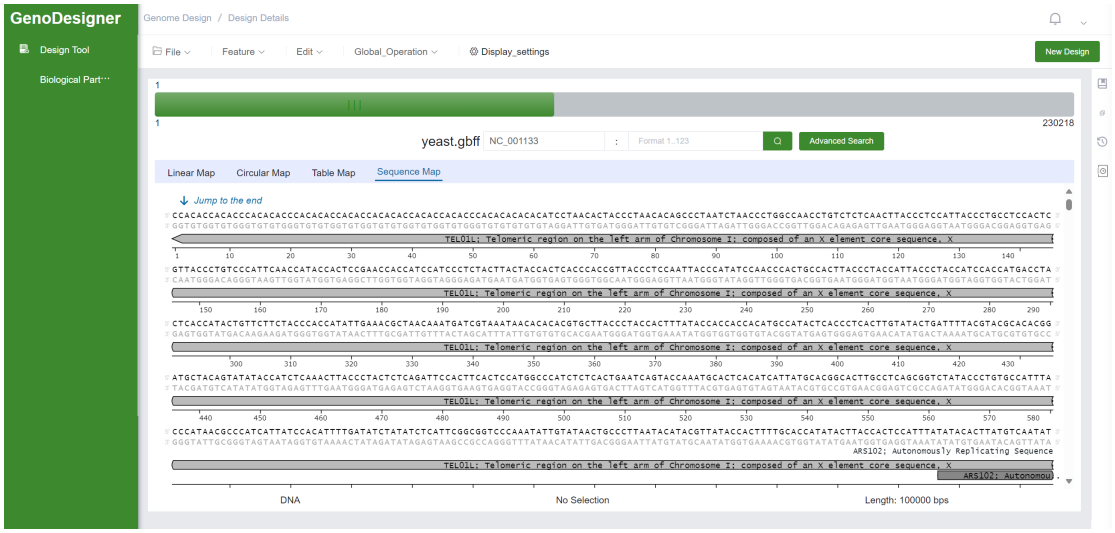

Fig. 2.5: Sequence map.

2.2 Design Information

Upon entering the design edit page, clicking on the right-hand menu "Design Information" allows you to view the design name, genome name, vector, and the annotation database used for the design. The design name, vector, and annotation database can be modified. After making the modifications, click the "OK" button to confirm the changes to the design information (Fig. 2.6).

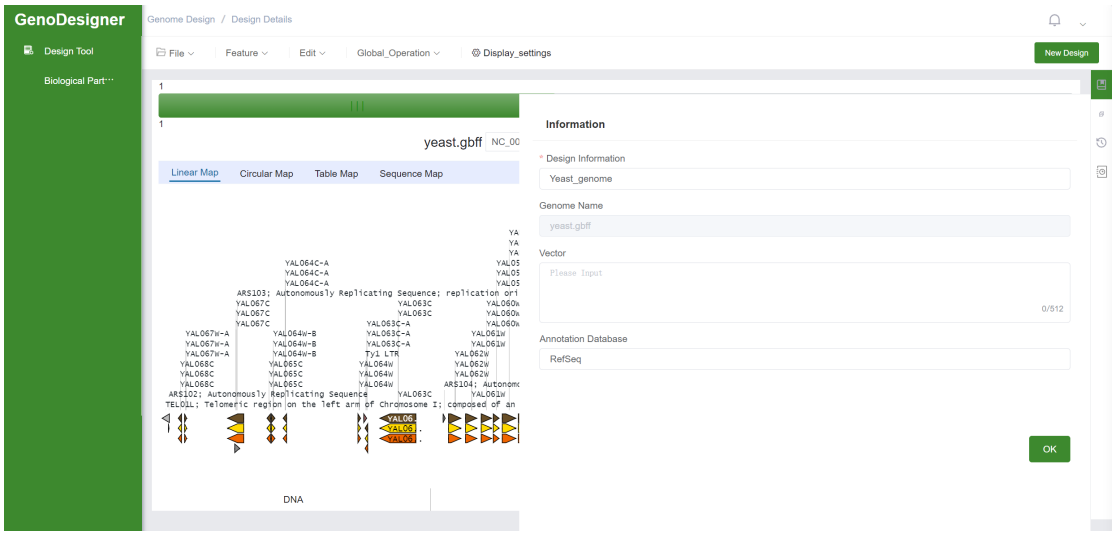

Fig 2.6 Design information.

2.3 Display Settings

For the sequence information of the chromosome, the default display shows 100,000 bp and 2000 features. Click on the "Display Settings" button in the options menu of the view to modify the displayed sequence length and the displayed features (Fig. 2.6, 2.7).

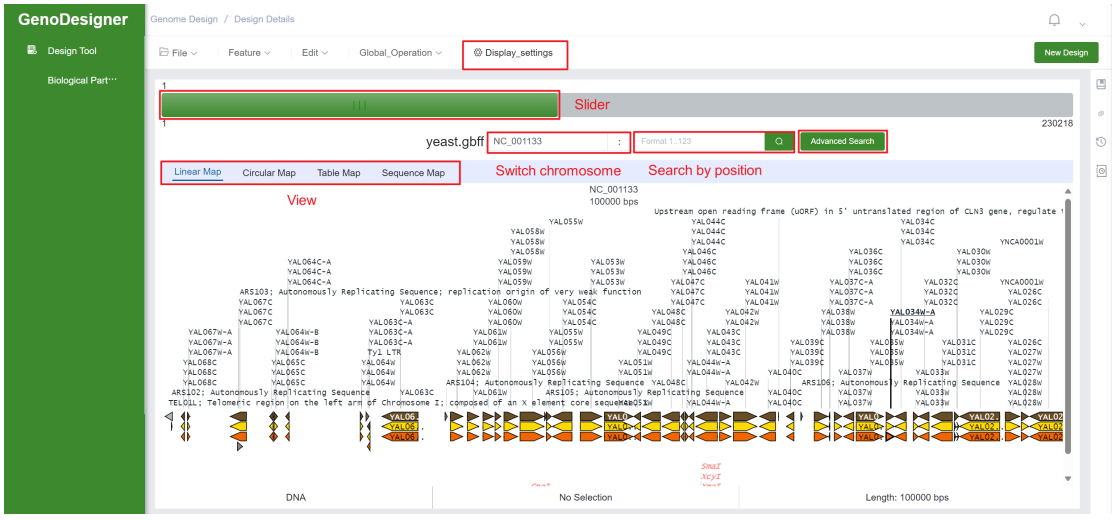

Fig. 2.6: Design interface.

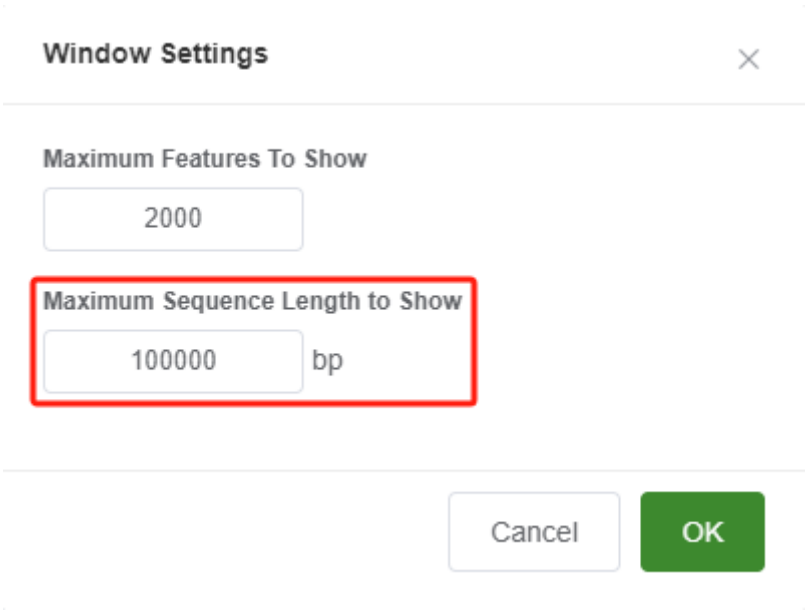

Fig. 2.7: Adjust display settings.

2.4 View Slider

The green slider can be dragged to move the view range of sequences (Fig. 2.6).

2.5 Feature Operations

The priority of displaying feature names in the software is ID > Name > locus_tag > label > others (according to attributes from annotations).

2.5.1 Import Features from Genbank file

This function allows users to detect and import common features from a given GenBank file. If the current sequence already contains such features (same type, location, strand, note), the redundant features won't be imported. To use this function, click "Feature" and "Import Features from Genbank Files", and select GenBank files to import (Fig. 2.8). The non-redundant features will be imported from the selected files. Currently, only the features that contain same sequences will be imported.

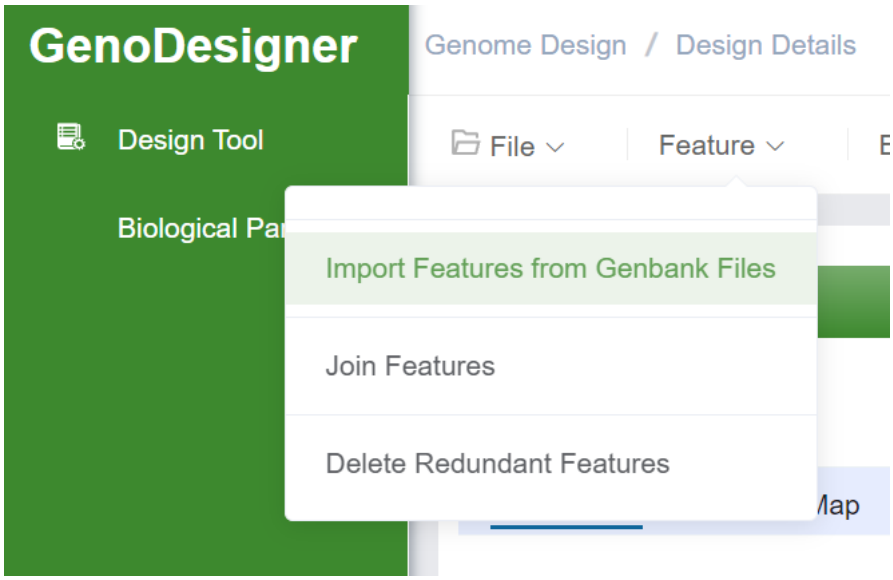

Fig. 2.8: Import Features from Genbank Files.

2.5.2 Delete Redundant Features

If there are redundant features in the chromosome, you can click “Features” and “Delete redundant Features”. A pop-up window will prompt the user to select what attributions are the same as the features to be deleted (Fig. 2.9). After confirming the deletion, the redundant features will be detected and removed.

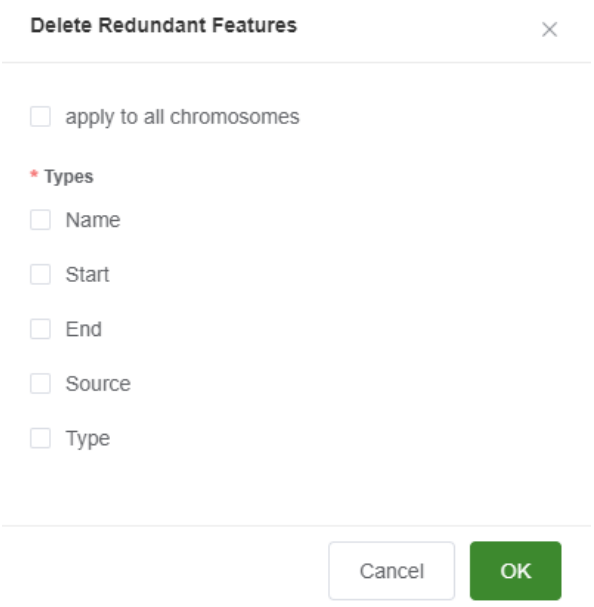

Fig. 2.9: Options of “Delete Redundant Features”

2.5.3. Join Features

Features imported from GFF3 files consist of simple features containing a single region. If certain elements in your genome contain multiple disjointed regions, such as genes with introns, we recommend processing these to form joined features to prevent unexpected errors. The “Join Feature” function can be utilized for this purpose. Within this function, the attributes “Parent” and “ID” are used to identify regions belonging to the same features. It is important to ensure that the features are imported from standard GFF files or Genbank files and that the features requiring joining must contain the attributes “Parent” and relevant unique identifiers like “ID” or “locus_tag”. Upon importing sequences and features, clicking the “Join Feature” button will remove the scattered features and generate relevant joined features (Fig. 2.10, 2.11).

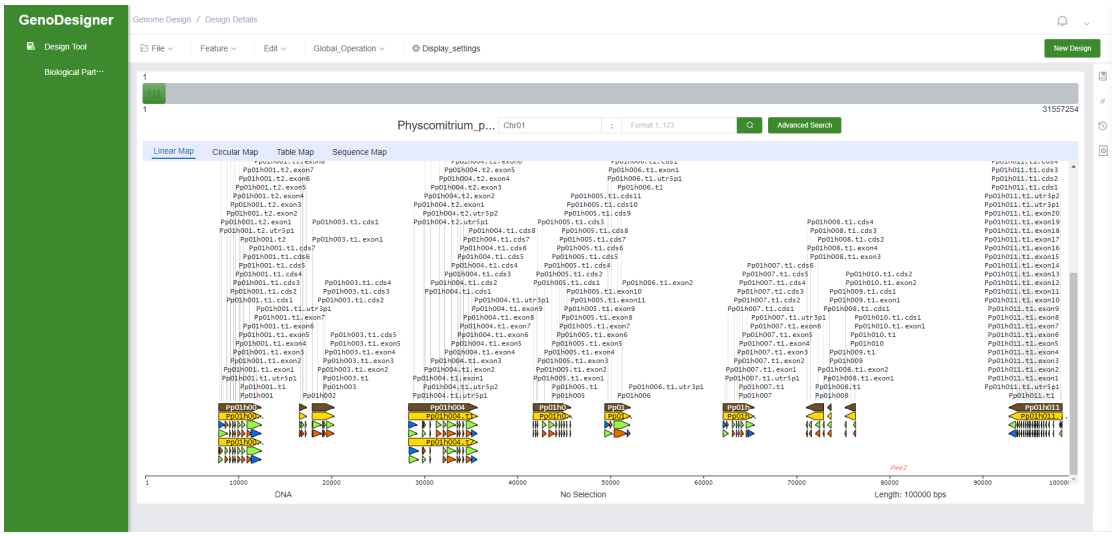

Fig. 2.10: Sequence with disjoint features.

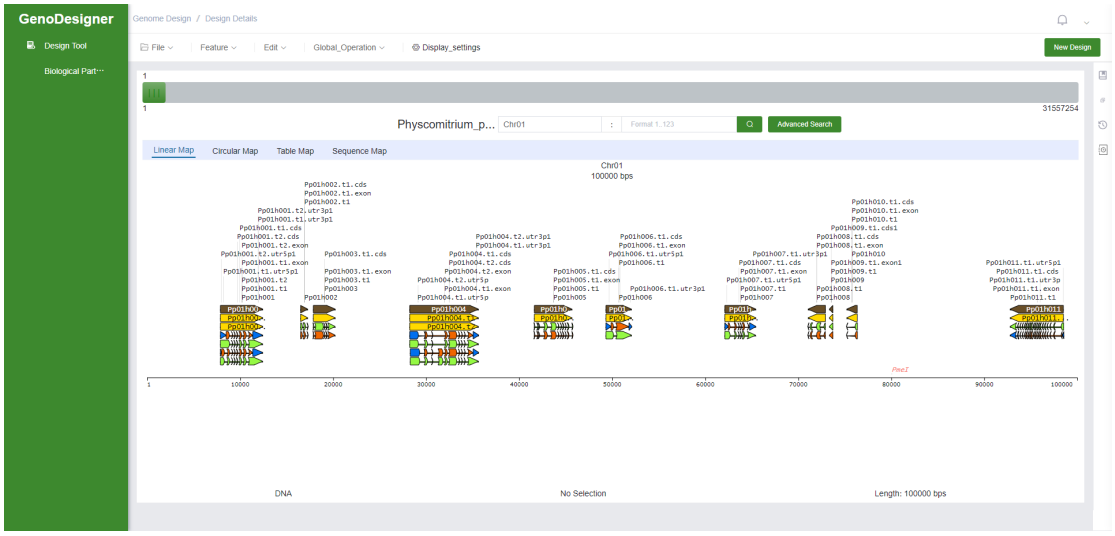

Fig. 2.11: Sequence with joined features.

2.6 Sequence & Feature Search

There is a search box above the view on the design edit page. The displayed search is a regular search (Fig 2.6). Clicking on "Advanced Search" will pop up an advanced search window (Fig. 2.6). The regular search is based on chromosome and gene sequence positions. The advanced search includes search by file, text, and feature name (Fig. 2.12).

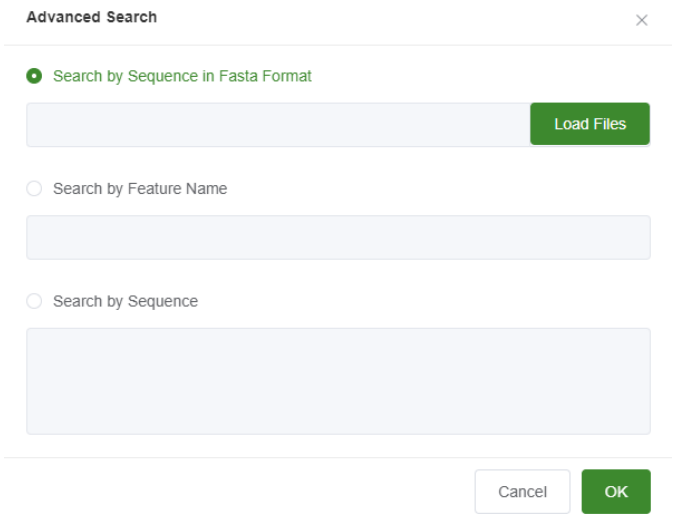

Fig. 2.12: Advanced search.

2.6.1 Regular Search

The regular search is divided into two parts. On the left side, you can select the chromosome to be searched. Clicking on the left side will pop up a dropdown menu where you can select the chromosome. On the right side, you can fill in the sequence position, which consists of two parts (start sequence position and end sequence position, 1-based) separated by "..". After filling in the positions, click the 🔍 button to perform the search, and the view will render the search results.

2.6.2 Search by File

After clicking "Advanced Search," select "Search by Sequence in FASTA Format" (Fig. 2.12). The system will search based on the sequence of the uploaded file. The file should only contain sequences in FASTA format (other formats are not allowed). The matched results are listed in the "Result" (Fig 2.13).

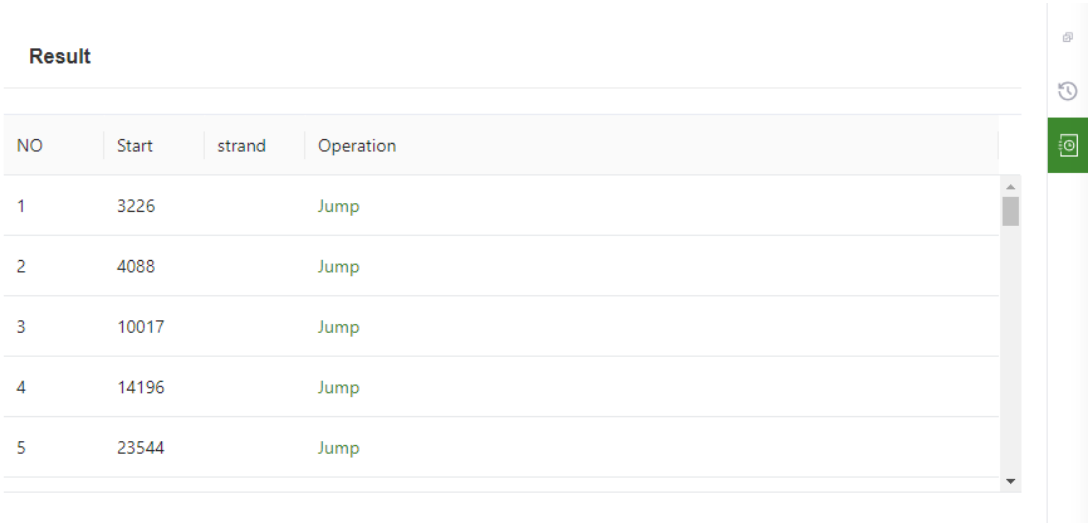

Fig 2.13 Search results

2.6.3 Search by sequence

Text Search: After clicking "Advanced Search," select “Search by Sequence”. The system will search based on the input sequence. Only DNA sequences can be entered for the search; other content is not allowed. The matched results are listed in the “Result” (Fig 2.13).

Note: DNA sequences only contain “atcgnrymkswhbvdATCGNRYMKSWHBVD”

2.6.4 Search by Feature Name

Feature Name Search: After clicking "Advanced Search," select “Search by Feature Name”. The system will search based on the input feature name. The matched results are listed in the “Result” (Fig 2.13)

Chapter 3

Basic Editing Operations

Cut, insert, replace, copy, paste, and other operations can be performed on the four rendered views. In the linear map, circular map, and sequence map, editing is done on the sequences, while in the table map, editing is done on the features, without modifying sequences. The rendering of the four types of views is synchronized. You can achieve these operations by clicking relevant buttons in “Edit” menu (Fig 3.1), right-clicking or shortcut keys. Right-clicking at a certain position in the linear map, circular map, or sequence map will bring up a menu bar with options for insertion and creating new feature. When a portion of the region is selected in the linear map, circular map, or sequence map, right-clicking will bring up a menu with options for replace, cut, and copy.

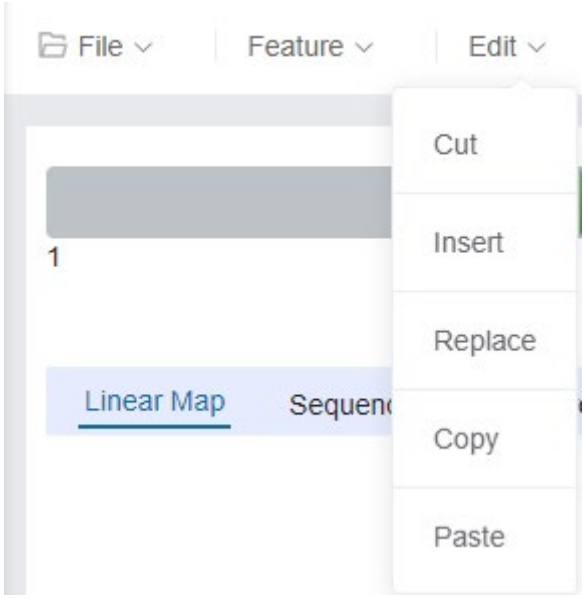

Fig. 3.1: The “Edit” menu.

3.1 Cut

After selecting a region and clicking “Edit” and "Cut," the selected sequence will be deleted, and copied to the clipboard. If a feature overlaps with but not included in the selected region, the part of feature within the region will be cut, leaving a truncated feature with a “.CUT” label, without removing the whole feature (Fig 3.2).

Fig. 3.2: Cut feature.

3.2 Insert

There are three ways to insert fragments. The first method involves inserting parts from the part library (Fig. 3.3). The second method entails inserting from uploaded files by clicking “Customize” and “Open Local File”, which must be in the “.fa”, “.fasta”, or “.fna” format and contain only the DNA sequences (Fig. 3.4). The third method involves inserting custom sequences by clicking “Customize” and inputting DNA sequences in the text box (Fig. 3.4). If you want to insert the sequence in the reverse direction, you can select “Reverse Complement”. If the position is inside a feature, the feature name will be added a “.INSERT” label (Fig. 3.5).

Note: DNA sequences only contain atcgnrymkswhbvdATCGNRYMKSWHBVD

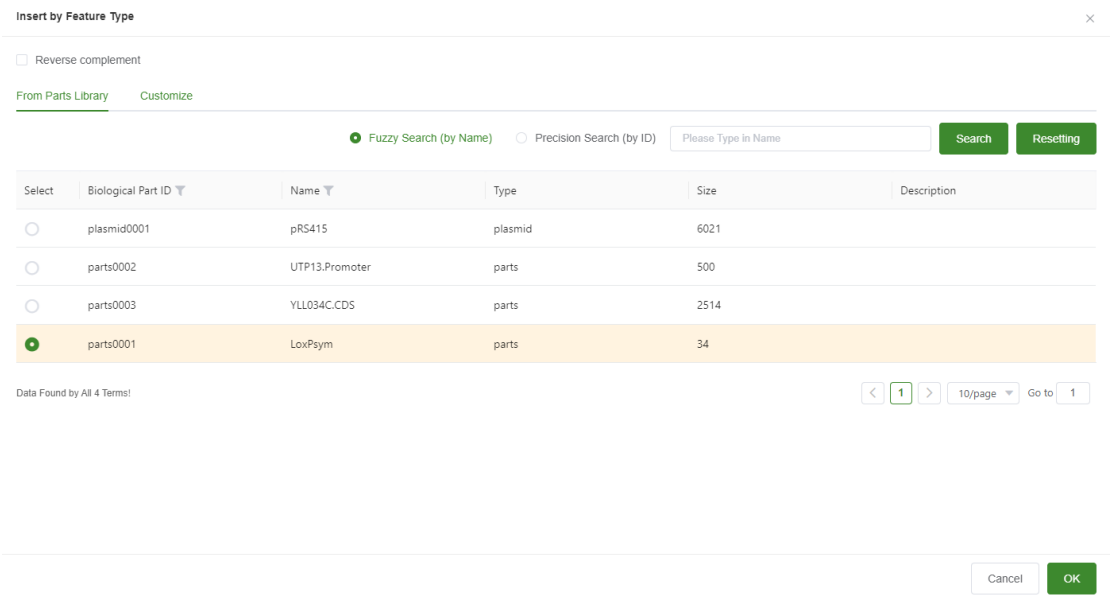

Fig. 3.3: Insert parts.

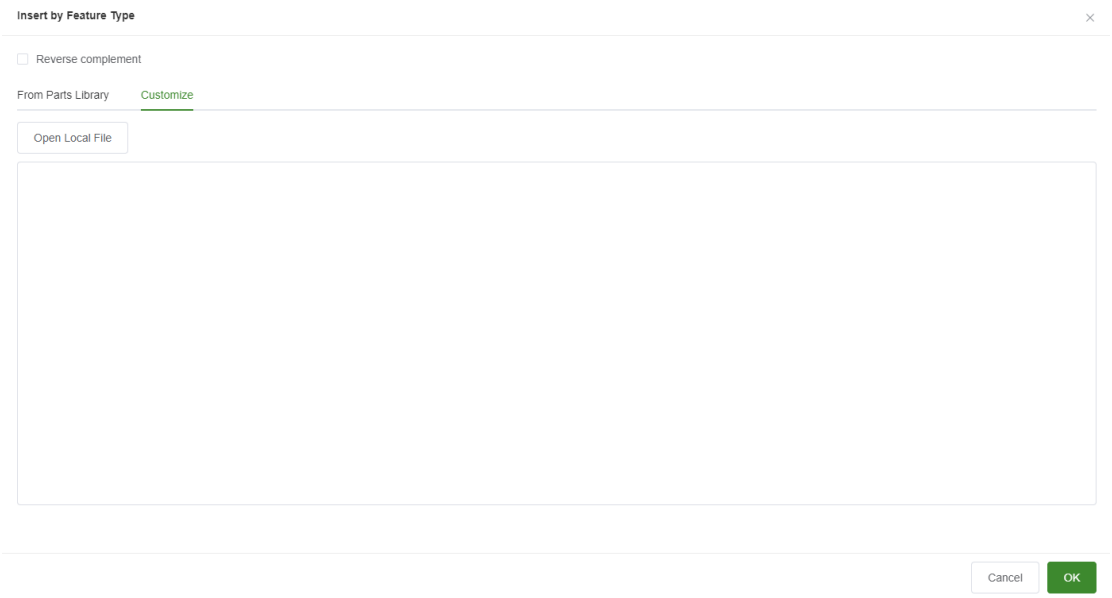

Fig. 3.4: Insert customized sequence.

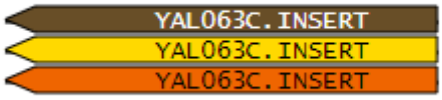

Fig. 3.5: Inserted feature.

3.3 Replace

After selecting a region and clicking “Edit” and “Replace” or right-clicking "Replace", a window includes the position, the original sequence, and a text box for entering the replacement DNA sequence will appear. After entering the replacement sequence, clicking "OK" will prompt the view renderer to replace the selected region (Fig 3.6). If the region overlaps with a feature, the feature name will be added a “.CUT.INSERT” label (Fig. 3.7).

Note: DNA sequences only contain atcgnrymkswhbvdATCGNRYMKSWHBVD

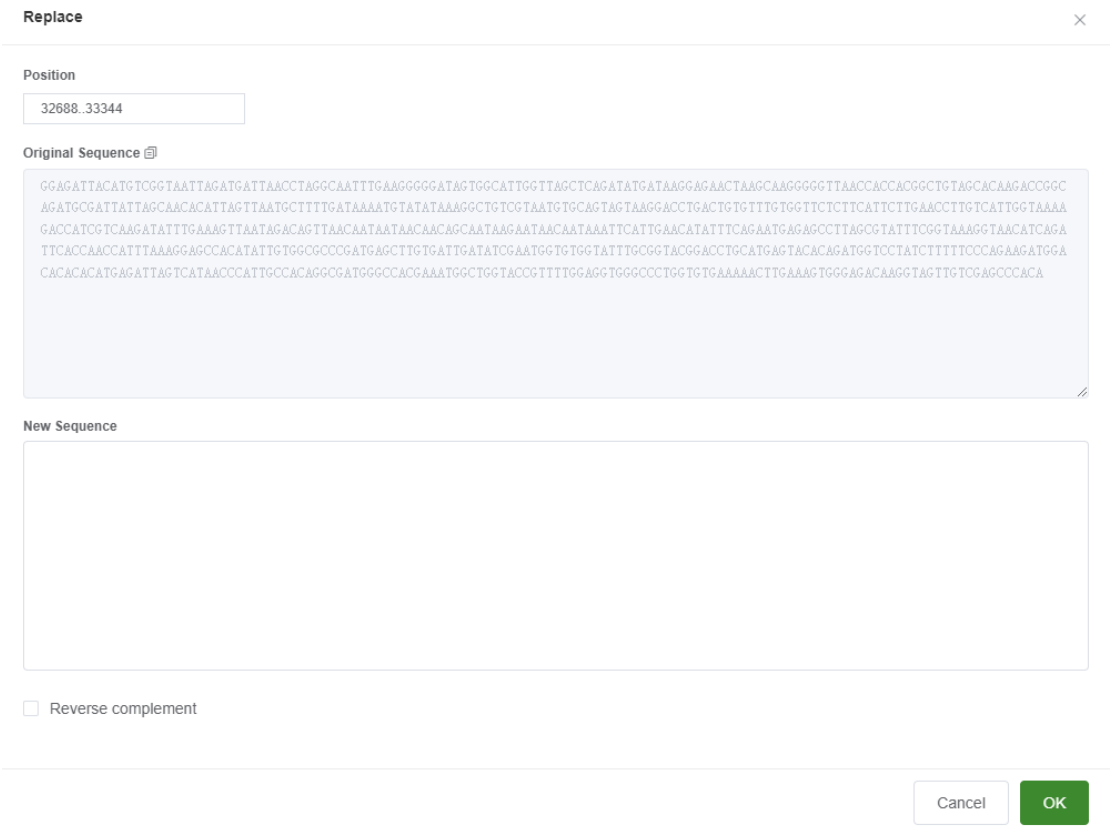

Fig. 3.6: Replace.

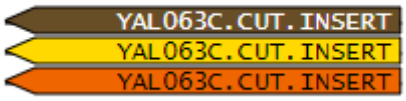

Fig. 3.7: Replaced feature.

3.4 Copy

After selecting a region and clicking “Edit” and “Copy” or press Ctrl + C, the selected sequence and features will be copied to the clipboard.

3.5 Paste

After selecting a position, clicking “Edit” and “Paste” or pressing Ctrl + V to insert the sequence and features previously copied to the clipboard onto the selected position.

3.6 New Feature

Clicking "New Feature" on any position or selected region of the sequence or clicking “New” in

table map will prompt a pop-up window. Filling in the feature's name, strand, type, position, and notes in the pop-up window and clicking "Save," the feature will be added (Fig 3.).

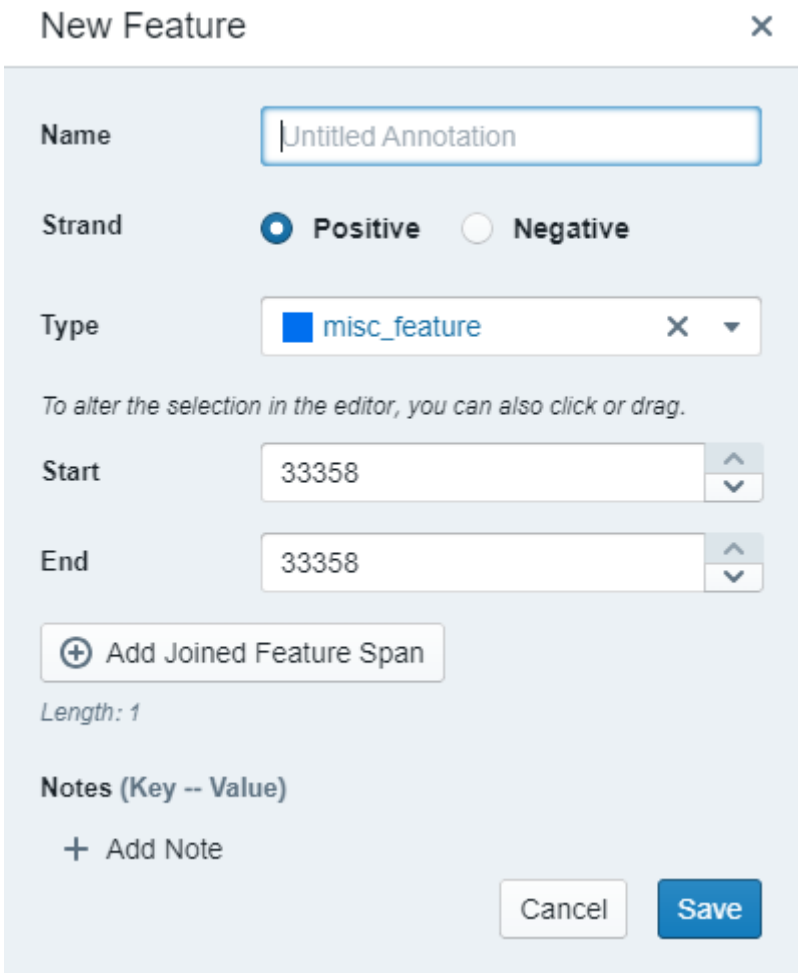

Fig. 3.8: Create new feature.

3.7 Edit Feature

The operations of the table map are all based on features, which do not affect sequences. There are four buttons in the table map. They represent four operations respectively, from left to right: New, Edit, Delete, and Remove Duplicate Features (Fig 3.9). You can also double-click the features to edit them.

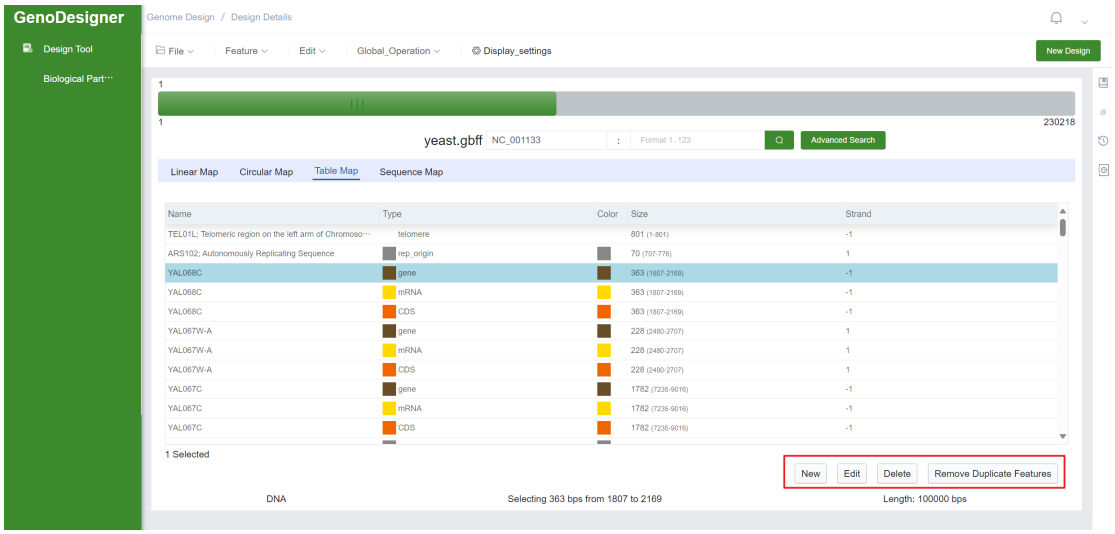

Fig. 3.9: Edit features in table map.

3.8 History

Every operation performed on sequences is recorded in the “History” (Fig. 3.10). The paste operation is recorded as an “insertion” and the delete operation is recorded as a “cut”. The copy operation is not recorded. The history records support version rollback, which means reverting to a historical version (for example: if there have been 10 edit operations in total and you roll back to the 7th edit operation, after the rollback, the history records for versions 8, 9 and 10 will be deleted).

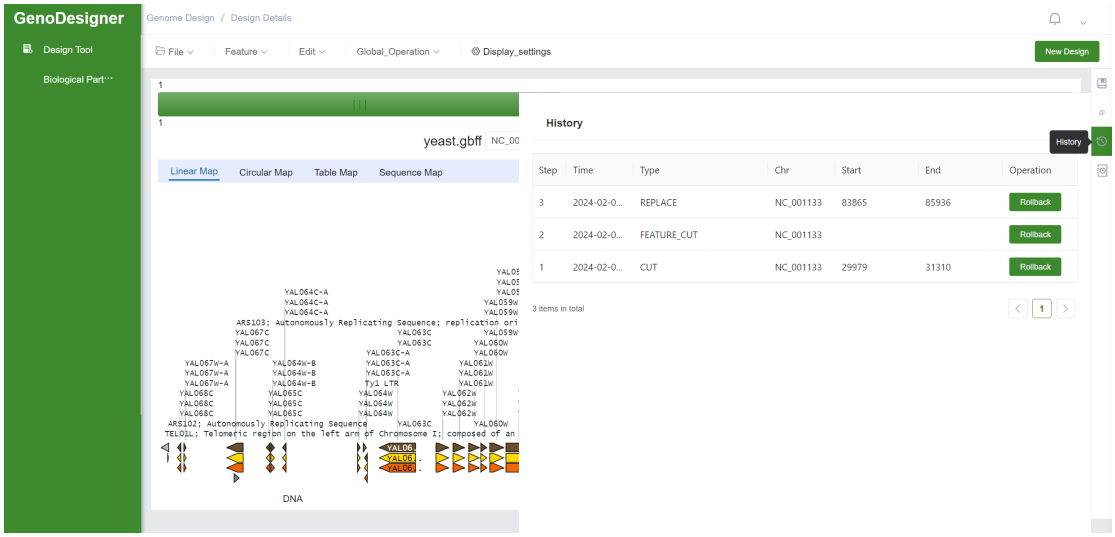

Fig. 3.10: History.

Chapter 4

Global Operations

Besides basic operations that allow users to modify genome sequences and features manually, GenoDesigner also provides many global operations for genome simplification and modification. Most global operation functions have an option “apply to all chromosomes”. While select it, all chromosomes in the design will be processed using the same arguments.

4.1 Open Editing File

This function allows users to accomplish batch editing operations using customized txt file. Each editing file contains a Tab-split table (column name ignore case) recording the chromosome name (Chr), start position (Start), end position (End), edit type (Edit_type), sequence after editing (Seq) and strand information (Strand). The “Edit_type” should be “CUT”, “INSERT” or “REPLACE”. If the “Ori_seq” column is provided in the file, GenoDesigner will check whether the sequences to be edit are the same as provided original sequence. This function recognize data by column names, and the order of the columns can be changed. All DNA sequences in the editing files are 5’ to 3’. The strand can be recorded as 1, -1. The positions in editing files are all 1-based, and represent the positions of sequences before editing by this function. This function can calculate the positions after changes in sequence length. If you want to add features on the inserted or replaced sequences, you can provide additional columns “Feature_name” and “Feature_type” and after insertion or replacement, new features will be added on the edited sequences according to information that you provided. After clicking “File” and “Open Editing File”, you can select whether the feature name should be changed (like adding “.CUT”, “INSERT” after original feature names) and select the editing file to upload (Fig. 4.1).

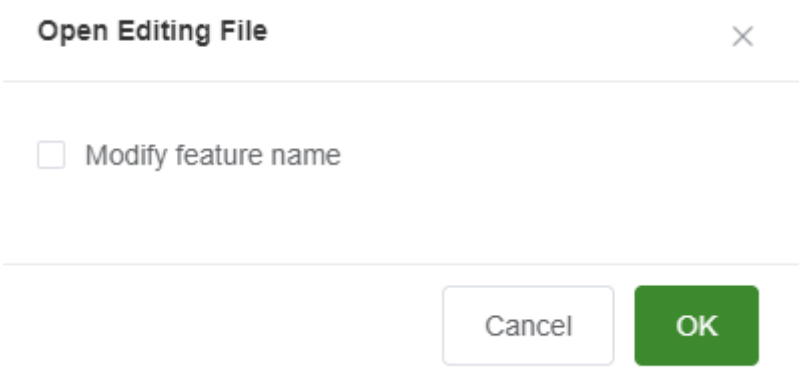

Fig. 4.1: The feature name modification option of “Open Editing File”

Example of editing file:

File 1 (edit the sequences only):

Chr	Start	End	Edit_type	Seq	Strand
Chr01	1	100	CUT	.	1
Chr01	200	200	INSERT	AAAAAAAAAAAAA	1
Chr01	301	400	REPLACE	CCCCCCCCCCCCCC	-1

File 2 (edit sequences and add features):

Chr	Start	End	Edit_type	Seq	Strand	Feature_name	Feature_type
Chr01	1	100	CUT	.	1	.	.
Chr01	200	200	INSERT	AAAAAAAAAAAAA	1	Insert_test	misc_feature
Chr01	301	400	REPLACE	CCCCCCCCCCCCCC	-1	Replace_test	misc_feature

4.2 Delete by Feature Type

If you want to remove all elements of a certain type (e.g. TEs) on the chromosome, click “Global Operation” and “**Delete by Feature Type**”. A pop-up window will prompt the user to choose what type of feature to remove and preserve (the feature types for selection are based on the current sequence), and multiple selections are supported (Fig. 4.2). If you select any type of feature to be preserved, you can also appoint upstream and downstream lengths to be preserved as well (e.g.

Selecting the "gene" and inputting values of 500 and 200 represent that during the removal of elements, any sequence located within the genes and their 500 bp upstream and 200 bp downstream will be retained). You can select “apply to all chromosomes” to process every chromosome in the current design with the same arguments. After confirming the deletion, the feature and its corresponding sequence will be deleted. If a feature overlaps with regions to be deleted and regions to be preserved, the sequence of the feature within the removal regions part will be cut, leaving a truncated feature with a “.CUT” label, rather than removing the whole feature. You can acquire reports summary and excel file containing positions being deleted in the “History” (Fig 4.3).

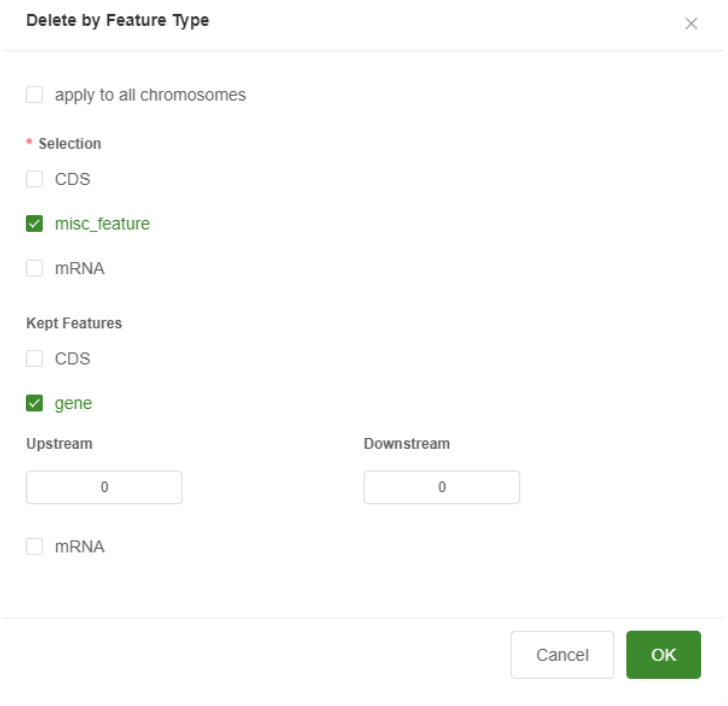

Fig. 4.2: Options of “Delete by Feature Type”.

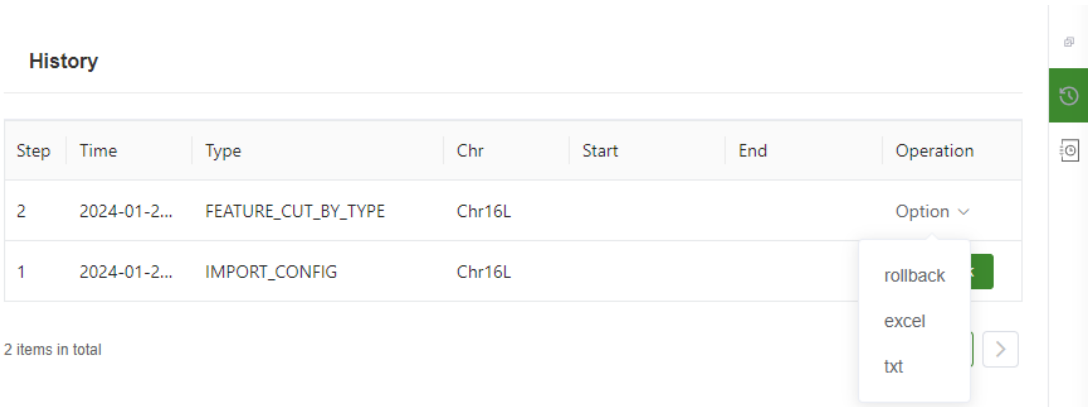

Fig. 4.3: Reports of “Delete Feature by Type”.

4.3 Truncate genome

Genomes contain many non-essential elements, and if you want to simplify the sequences (e.g. keep genes, tRNAs, centromeres, etc., and remove the others), click “Global Operation” and “Truncate genome”. A pop-up window will prompt the user to choose what type of feature with their upstream and downstream lengths to be preserved, and multiple selections are supported (Fig 4.4). You can select “apply to all chromosomes” to process every chromosome in the current design with the same arguments. After confirming the truncation, the sequences out of the selected regions will be deleted. If a feature overlaps with regions to be deleted and regions to be removed, the sequence of the feature within the removal regions part will be cut, leaving a truncated feature with a “.CUT” label, rather than removing the whole feature. You can acquire reports summary and excel file containing positions being edited in the “History” (Fig 4.5).

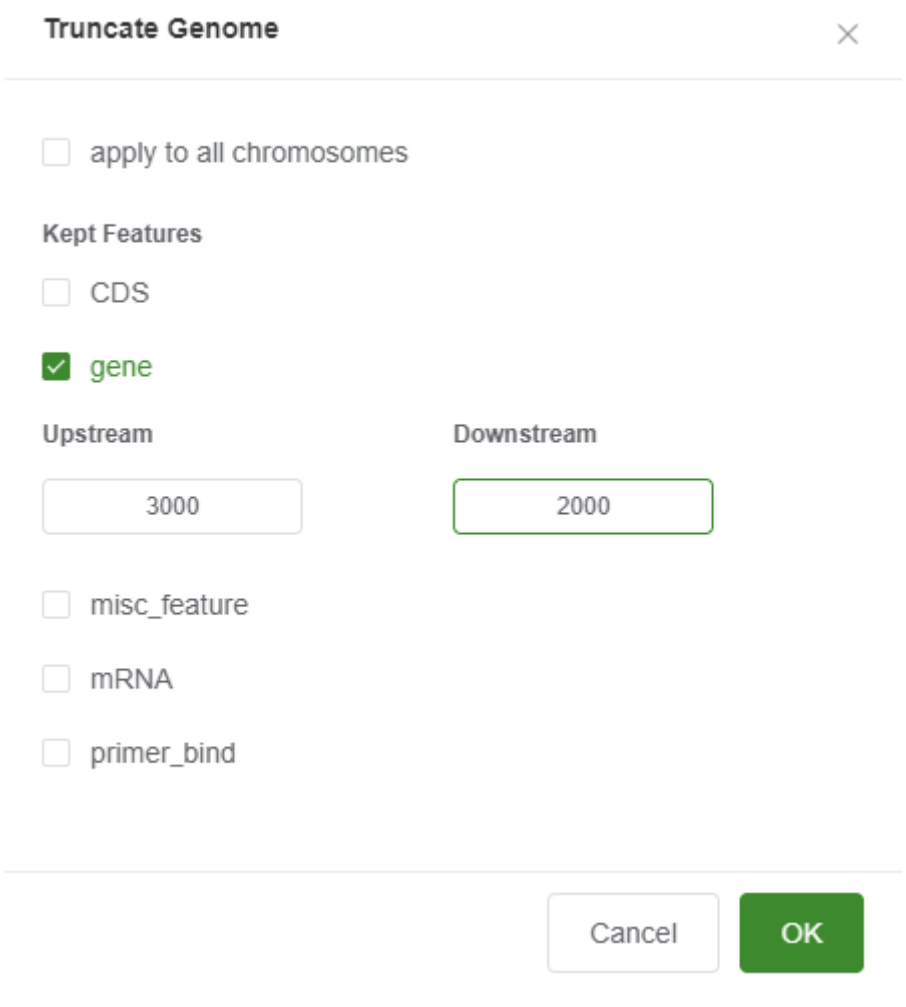

Fig. 4.4: Options of “Truncate Genome”.

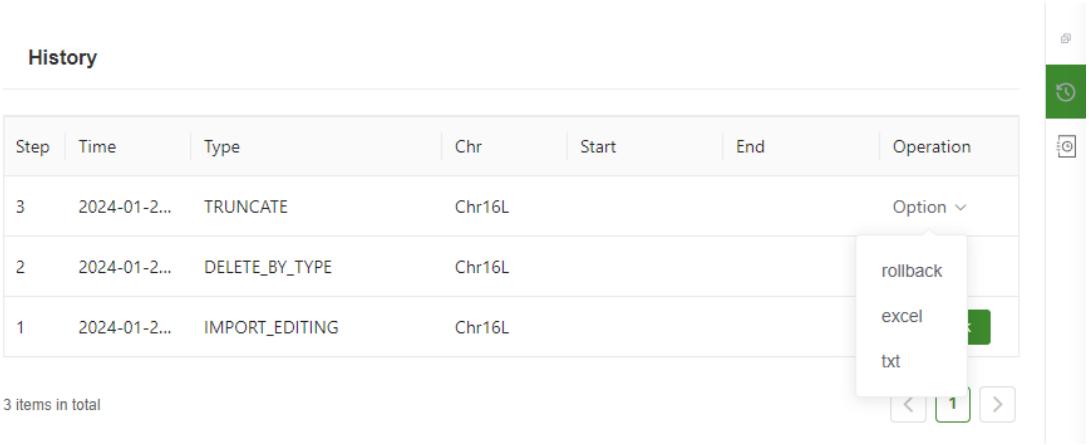

Fig. 4.5: Reports of “Truncate Genome”.

4.4 Replace Stop Codons

Reducing codon usage is common in synthetic genome design. If you want to replace one kind of stop codon with another (e.g. “TAG” to “TAA”) or even unify them (e.g. “TAG” and “TGA” to “TAA”), click “Global Operation” and “Replace Stop codons”. A window will appear to allow user to select which or which two stop codons should be diminished and which should be used for replacement (Fig 4.6). You can select “apply to all chromosomes” to process every chromosome in the current design with the same arguments. After confirming the truncation, the selected stop codons will be replaced. For genes containing multiple transcripts, the replacement of stop codons may affect the other CDSs. Therefore, while using GenoDesigner to replace stop codons, the codons overlap with other coding regions won’t be replaced, and you can get the report text file and excel file containing information of codons replaced and not replaced in the “History” (Fig 4.7). The excel file contains 3 sheets: replace_data (information of replaced stop codons), replace_ERR (information of stop codons that overlap with other coding regions), stop_codon_ERR (information of stop codons that are not TAG, TGA or TAA)

Note: This function requires joined CDS annotations, that is, joined features if there are introns in genes.

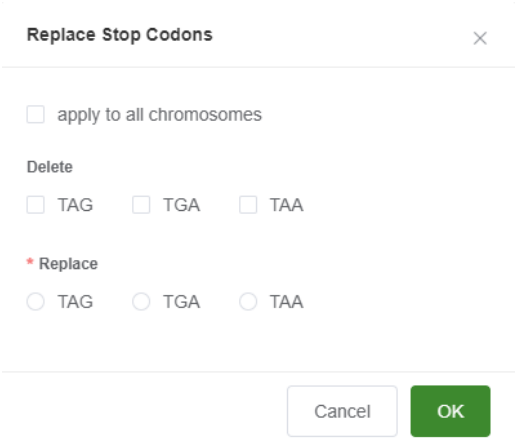

Fig. 4.6: Options of “Replace Stop Codons”.

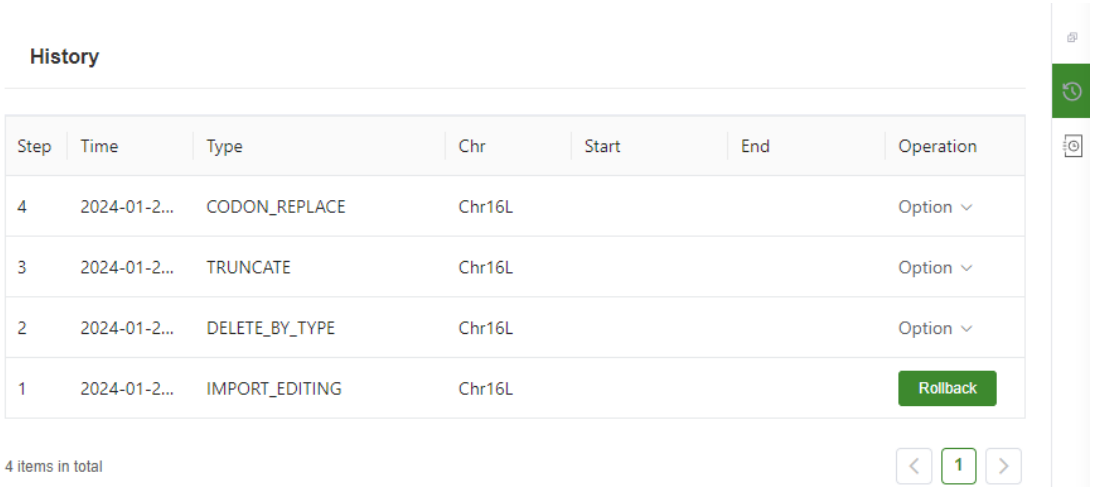

Fig. 4.7: Reports of “Replace Stop Codons”.

4.5 Insert by Feature Type

The “Insert by Feature Type” function allows you to insert any element that you provide globally based on feature type and relevant positions (e.g. loxPsym sequences to form SCRaMbLE system). Click “Global Operation” and “Insert Element”, and a window will appear to allow you to input the feature name and type of the element to be inserted (Fig. 4.8). You can select “apply to all chromosomes” to process every chromosome in the current design with the same arguments. The inserted elements will be labeled as features based on the information that you provide. Below the “Choose”, you can select the feature type to insert, and appoint the position by selecting upstream and downstream as well as input numbers. The numbers represent the relevant distance to the feature start and end. If numbers are greater than 0, the elements will be inserted outside the features. If the

numbers are less than 0 (e.g. -3), the elements will be inserted inside the features. If the numbers are equal to 0, the elements will be inserted right beside the features. Below the “Feature to Pretect”, you can select the feature type not to be modified. The sequences to insert can be selected in the parts database or customized. For example, if you want to insert 6xHis-Tags on the N and C terminus of every CDS, input “6xHis-Tag” in “Feature Name”, input “CDS” in “Feature Type”, select “CDS” in “Feature to Insert”, select “Upstream”, input “-3” under “Upstream”, select “Downstream”, input “-3” under “Downstream”, select “yes” at “adjust sequence direction by features”, select “Customize”, input “CATCACCATCACCATCAC”, and click on “OK”. The 6xHis-Tag sequences will be inserted downstream of the start codon and upstream of the stop codon of every CDS. You can acquire reports summary and excel file containing positions being inserted in the “History” (Fig. 4.9).

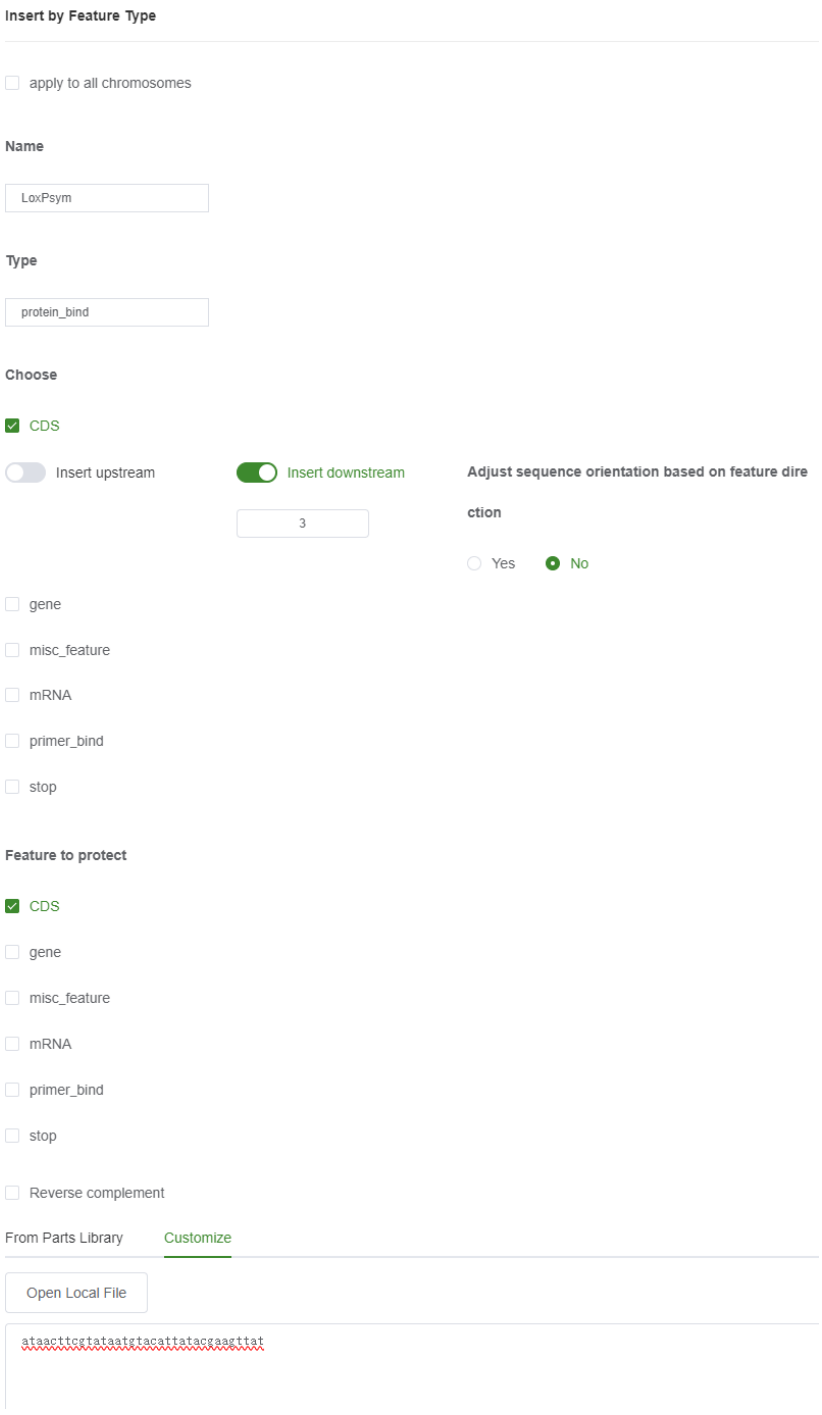

Fig. 4.8: Arguments of “Insert by Feature Type” for introducing SCRaMbLE system.

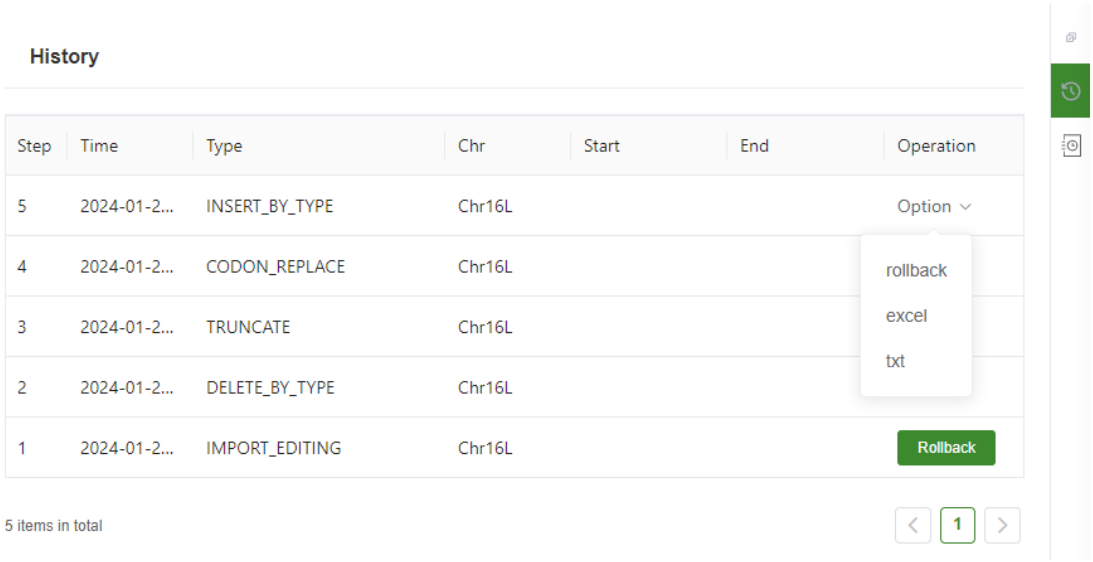

Fig. 4.9: Reports of “Insert by Feature Type”.

Chapter 5

Design Management

5.1 Design List

Click “Design Tools” on left sidebar, the design list will be loaded based on the creation time in descending order. Additionally, the design list page includes other operations querying, editing, deleting, downloading, cloning a design, and saving as a template (Fig. 5.1).

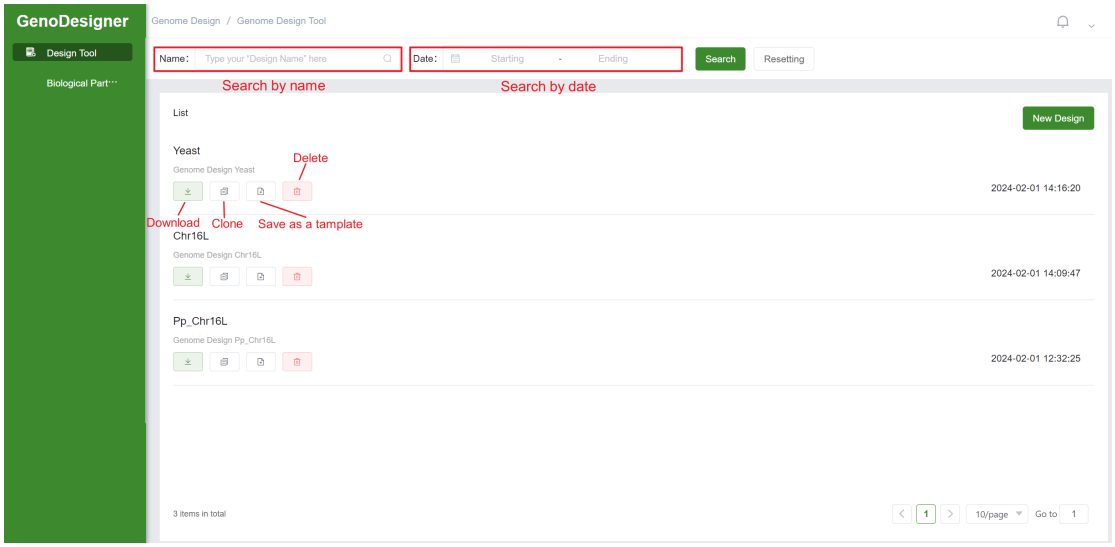

Fig. 5.1: Interface of design list.

5.2 Design query

Fill in the design name at the top of the design list and click on 'query' for the update time. The design list information will be queried according to the specified filter criteria for the corresponding design information (Fig. 5.1).

5.3 Save design as a template

Once you upload a genome or complete a design, you can select it in design list, and click on “Save as Template” (Fig. 5.1). After clicking, a dialog will appear to enter the template name (which cannot be the same as any existing templates). After entering the template name, click “OK”. Upon clicking,

a pop-up window with the message “Save successfully” will appear, indicating the successful addition of the new template.

5.4 Delete a design

Clicking “Delete” under the target design will prompt a confirmation window for deleting the design. Click “OK” to confirm (Fig. 5.1). Upon confirmation, a pop-up window with the message “Deletion completed” will indicate the successful deletion of the design.

5.5 Clone a design

Clicking “Clone” under the target design will prompt a window for entering the cloned design name (Fig. 5.1). Upon confirmation, a pop-up window with the message “Add successfully” will indicate the successful cloning of the design.

5.6 Download design

Clicking “Download” under the target design will prompt a window for selecting the files to be packaged and downloaded (Fig. 5.1). This will download the files you selected to your local PC in ZIP compressed format (Fig. 5.2).

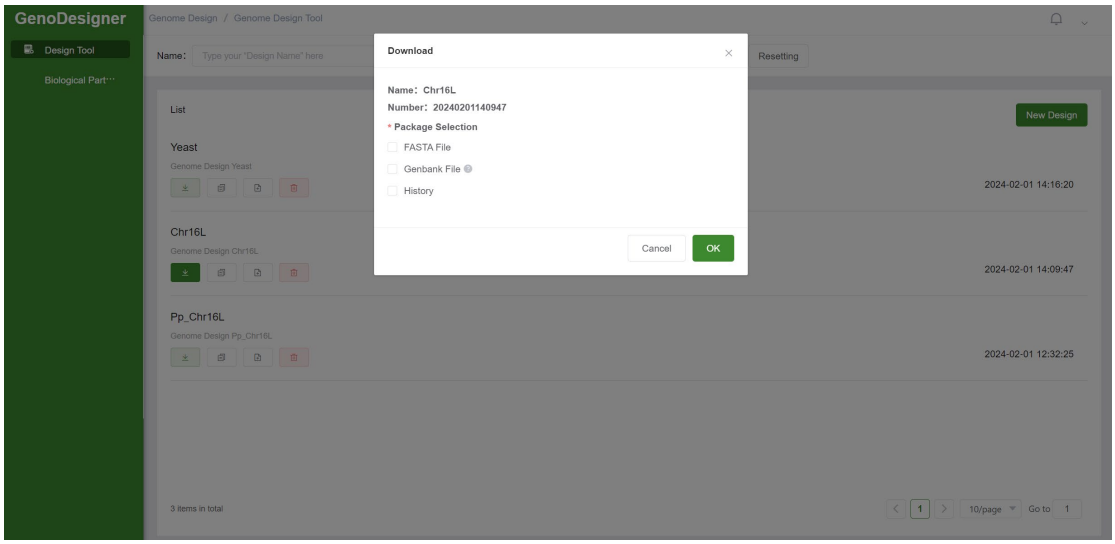

Fig. 5.2: Download design.

Chapter 6

Biological Parts Management

6.1 Parts Library Interface

Upon entering the system, click on the left-hand menu "Biological Parts Library." The parts list will be displayed, including the part ID, part name, part length, description, creation time, and operations (Fig 6.1).

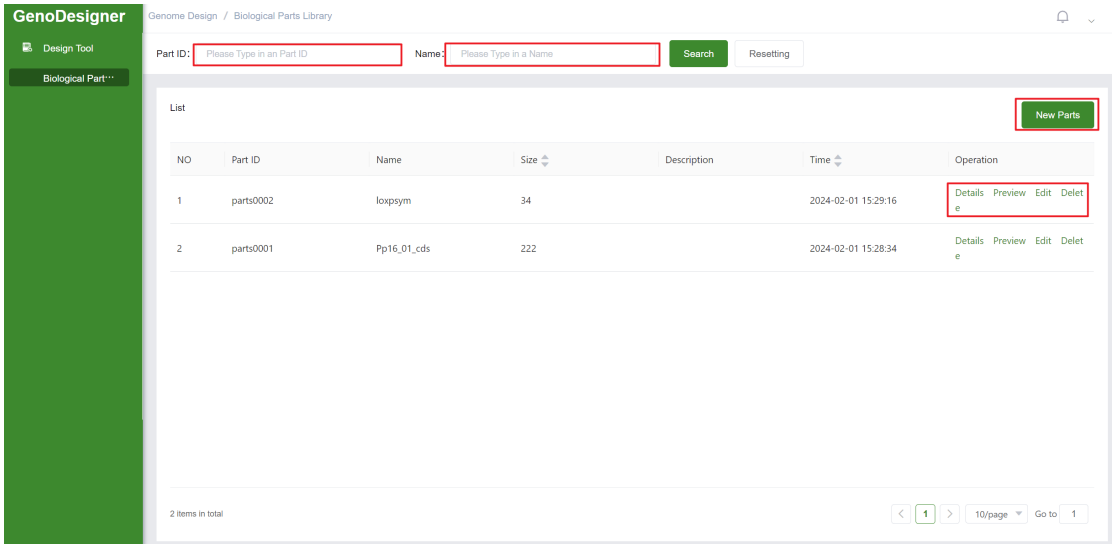

Fig 6.1 “Biological Part Library” interface

6.2 Manage Parts

6.2.1 Create New Part

Click on "Create New Part". A pop-up window will appear, allowing the creation of a new part by inputting the name, type, description, and sequence (Fig 6.2). After filling in the information, click the "OK" button, and a notification will pop up to inform the user of the successful addition.

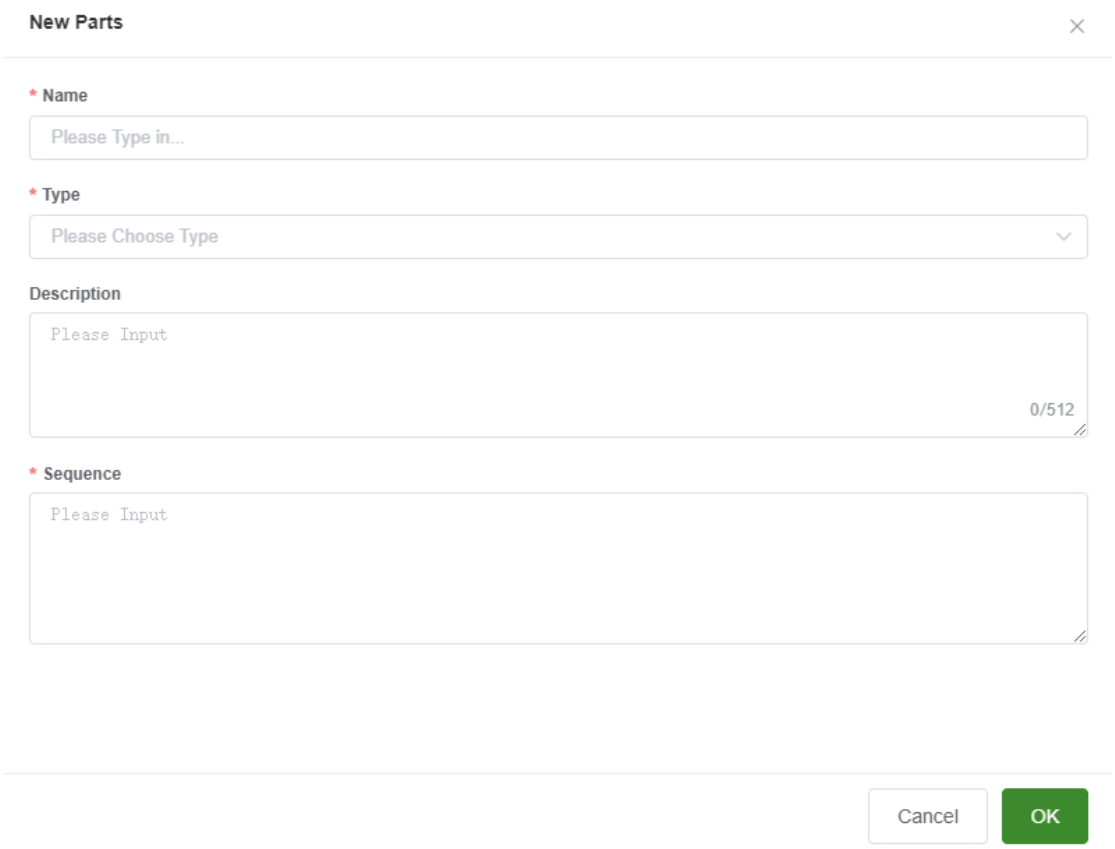

Fig. 6.2: Create new parts

6.2.2 Parts Details

Select a part and click on "Details", a pop-up window will appear, where you can view the information of the part (unable to modify). After finishing viewing, clicking the "Close" button will close the pop-up window .

6.2.3 Preview Parts

Select a part and click on "Preview", a pop-up window will appear, where you can view the sequence and structure of the part (unable to modify) and download. After viewing, clicking the "Close" button will close the pop-up window.

6.2.4 Edit Parts

Select a part and click on "Edit", a pop-up window will appear, where you can edit the information and sequence of the part. After editing, click the "OK" button, and a notification will pop up to

inform the user of the successful editing.

6.2.5 Delete Parts

Select a part and click on "Delete", a pop-up window will appear, where you can select “OK” to delete the part or “Cancel”.