Example

Design of synthetic Chr16L of *P. patens*

1 Start a new design

Click “Design Tool” on the left sidebar and click on “New Design” on the right (Fig. 1). Enter the design name and choose “Blank Design”. After entering the design name, like “Pp_Chr16L”, click “OK”, and you will be directed to the design page (Fig. 2).

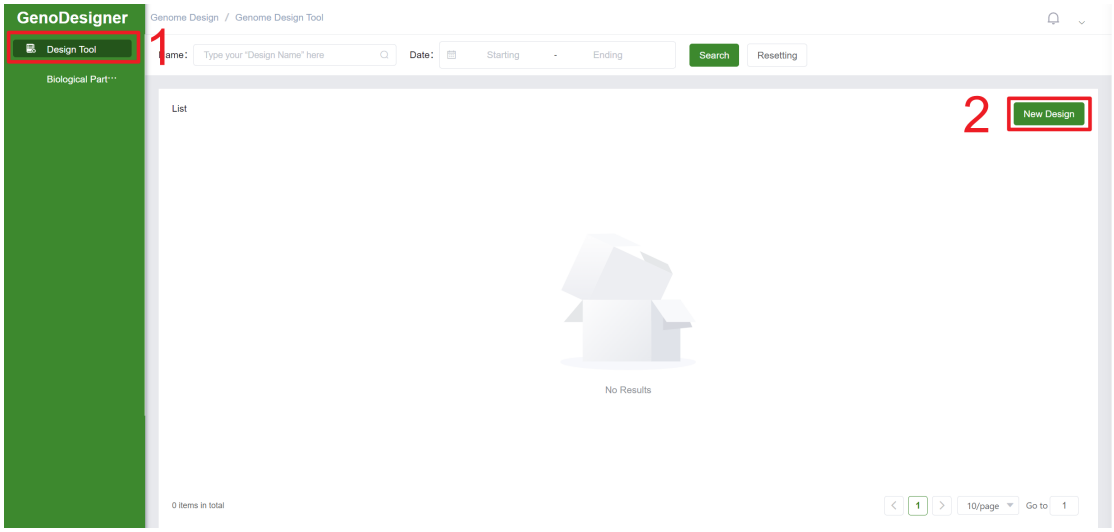

Fig. 1: Interface of Design Tool.

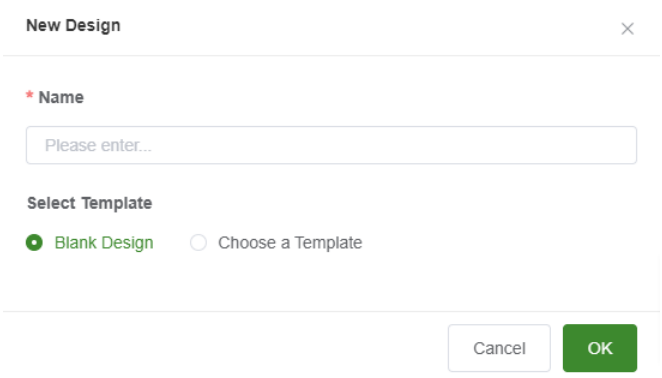

Fig. 2: Create new design.

2 Upload the genome

You can download the example Genbank file “Pp.Chr16L.gb” from Github (https://github.com/WenfeiY/P.patens_geno_design). Click on “File”, “Open Files” and select file

“Pp.Chr16L.gb” in the file upload dialog (Fig. 3). After opening the file, you can browse information in this sequence file. The default length to display is 100 Kb, and you can zoom in or out by adjusting “Maximum Sequence Length to Show” in the “Display_settings” (Fig. 4, 5). GenoDesigner supports four types of views: linear map, circular map, table map (feature list), and sequence map (Fig. 4).

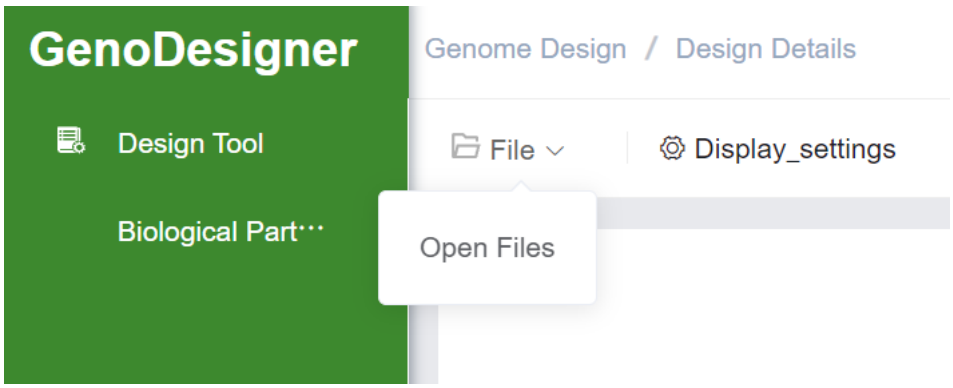

Fig. 3: Open files.

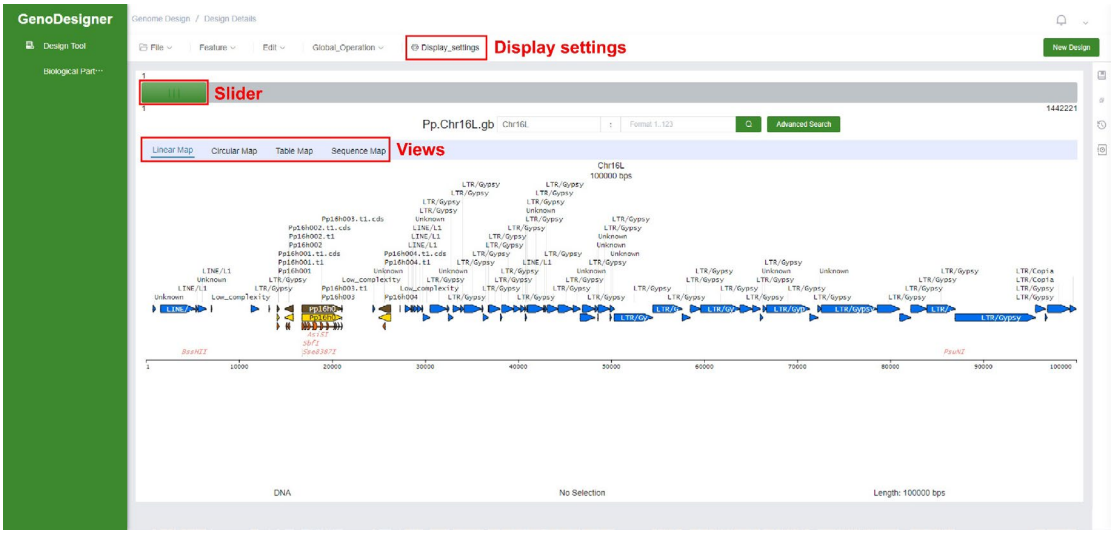

Fig. 4: Sequence interface.

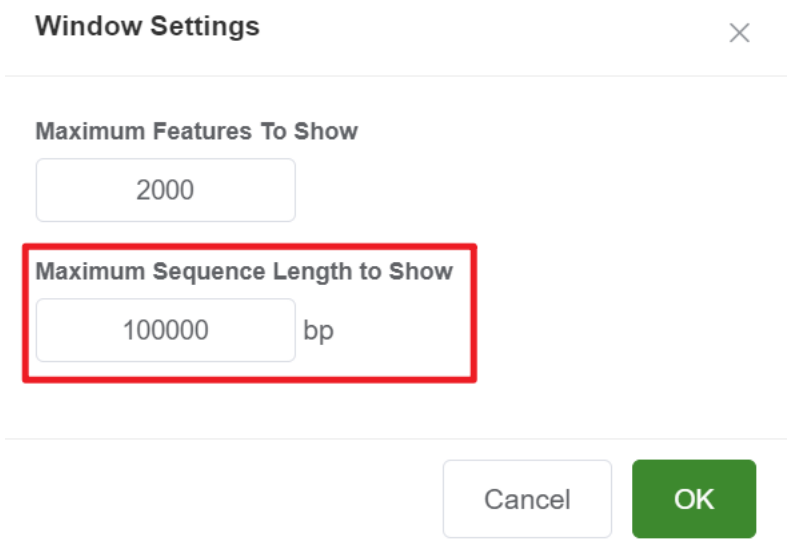

Fig. 5: Adjust display settings.

3 Edit your genome

GenoDesigner provides many global operation functions, allowing users to generate genome-wide modified sequences (Fig. 6).

The design of *P. patens* Chr16L includes the removal of all the TEs, truncation of intergenic regions, introduction of PCRmarks, unification of stop codons, and incorporation of the SCRaMbLE system. The synthetic Chr16L can be gained through the following steps.

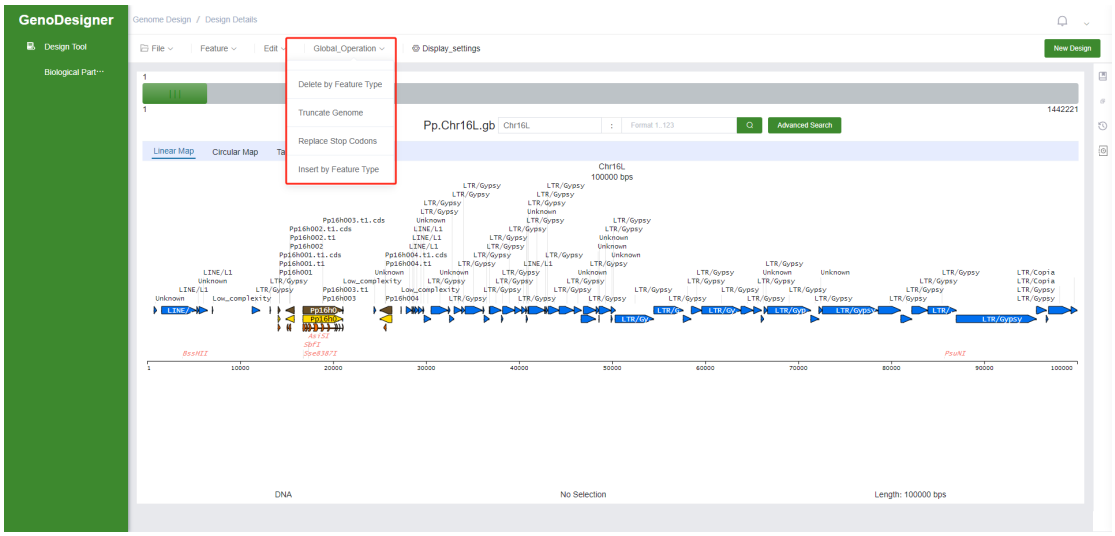

Fig. 6: Global operations.

3.1 Open Editing File (introduce PCRmarks)

Firstly, download the file “Pp.Chr16L_PCRmark.txt” containing information on PCRmarks from Github (https://github.com/WenfeiY/P.patens_geno_design). Click on “File”, “Open Editing File”, “OK”, and select “Pp.Chr16L_PCRmark.txt” to introduce PCRmarks (Fig. 7). The relevant sequences on chromosome will be replaced and the annotations of PCRmarks will be added to the regions.

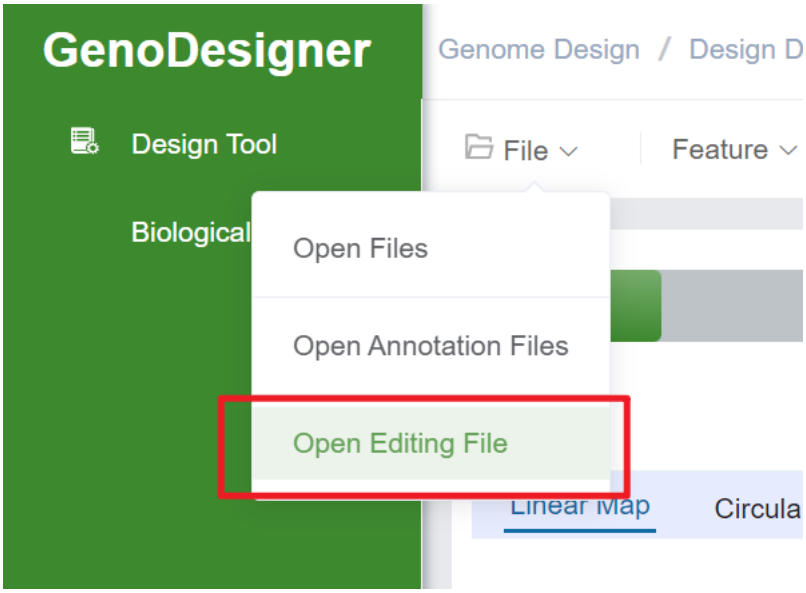

Fig. 7: Introduce PCRmarks through “Open Editing File”.

3.2 Delete by Feature Type (remove TEs)

The “Delete by Feature Type” function allows you to delete any kind of feature in the current genome, and to avoid the deletion of other elements, you can select the feature type and input upstream / downstream length to protect these regions from deletion. For synthetic Chr16L design, at this step, select “misc_feature” (all TEs are recorded as “misc_feature” in this sequence file) to be deleted and “gene” to be protected, and click on “OK” (Fig. 8). All “misc_feature”, except in the protected regions, will be removed.

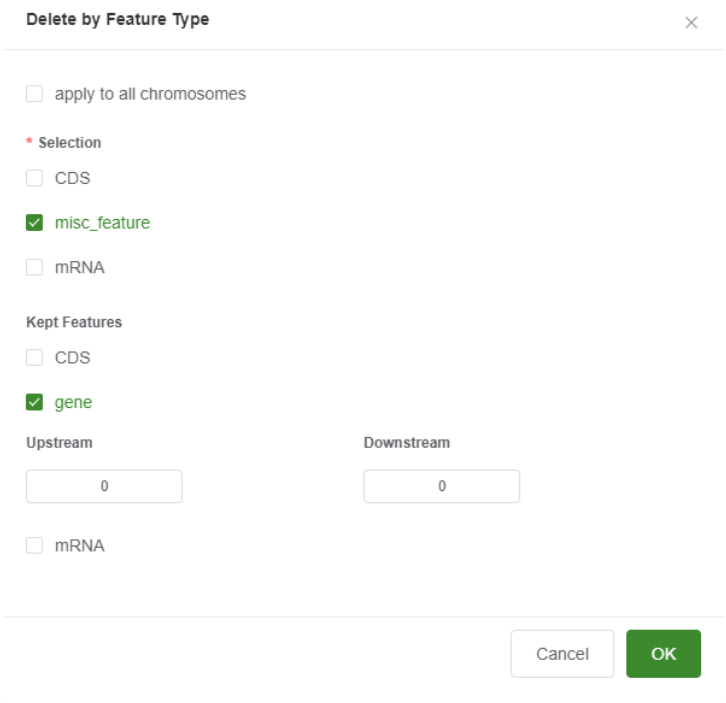

Fig. 8: Arguments of “Delete by Feature Type” for removal of all TEs.

3.3 Truncate Genome (truncate intergenic regions)

The “Truncate genome” function allows you to preserve features with their upstream and downstream sequences in the current genome and remove the others. For synthetic Chr16L design, at this step, select “gene” to be preserved, input “3000” for the upstream length, “2000” for the downstream length and click on “OK” (Fig. 9). All regions extended selected regions will be removed.

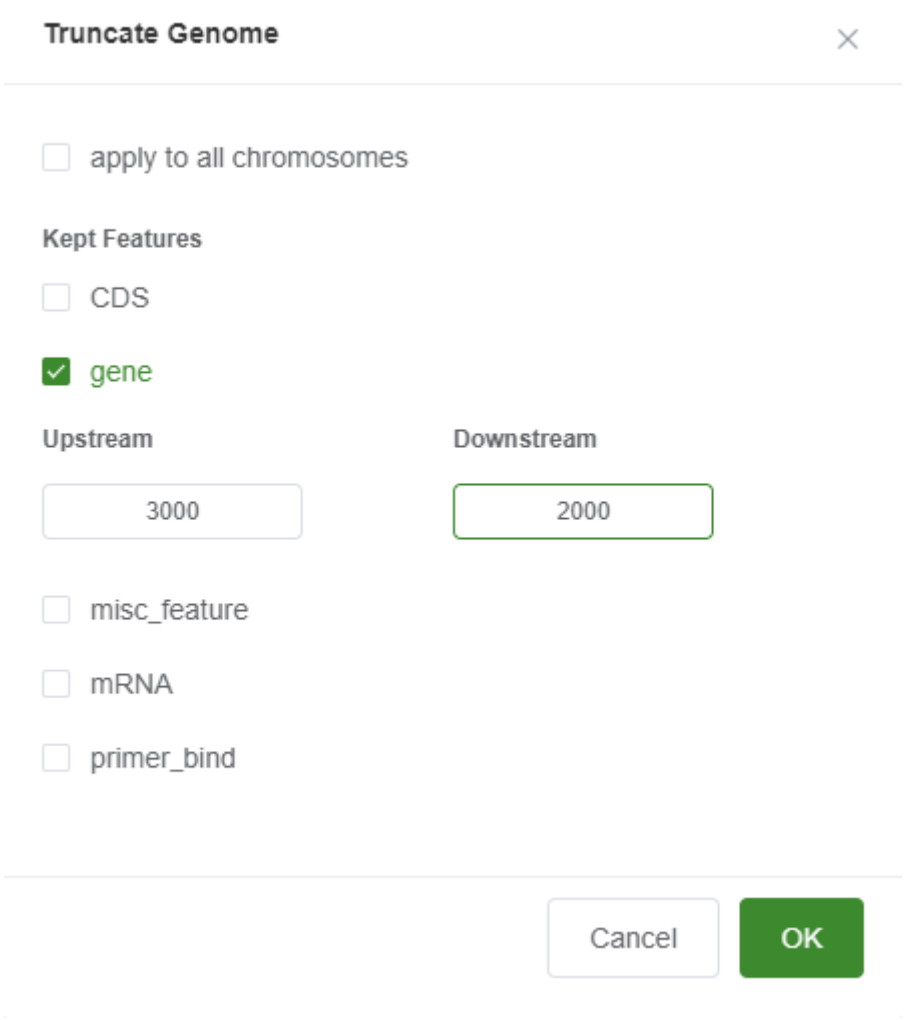

Fig. 9: Arguments of “Truncate Genome” for truncating intergenic regions.

3.4 Replace Stop codons (unify stop codons)

The “Replace Stop codons” function allows you to replace selected stop codons with others in all CDSs. Before you use this function, please make sure that all CDSs with introns are recorded as **joined features**. You can also use the “Join Features” function (See Help: Chapter 2.5.3). For synthetic Chr16L design, at this step, select “TAG” and “TGA” to be swapped, select “TAA” for replacement, and click on “OK” (Fig. 10). All “TAG” and “TGA” stop codons will be replaced by “TAA”. Stop codons that overlap with other CDSs will not be swapped to avoid changes in protein sequences.

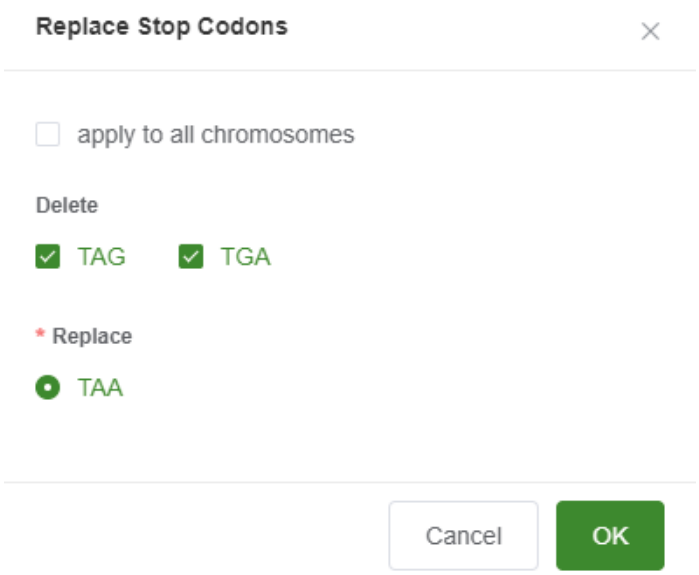

Fig. 10: Arguments of "Replace Stop Codons" for replacing TAG and TGA to TAA.

3.5 Insert by Feature Type (incorporate SCRaMbLE system)

The "Insert by Feature Type" function allows you to insert any element that you provide globally based on feature type and relevant positions. The inserted elements will be labeled as features based on the information that you provide. For synthetic Chr16L design, at this step, input "LoxPsym" in "Feature Name", input "protein_bind" in "Feature Type", select "CDS" in "Feature to Insert", select "Downstream", input "3" under "Downstream", select "CDS" in "Feature to Protect", select "Customize", input "ataacttcgtataatgtacattatacgaagttat", and click on "OK" (Fig. 11). The loxPsym sequences will be inserted at 3 bp downstream of every CDS, except those positions that overlap with other CDSs. As a result, only one loxPsym will be introduced for each gene.

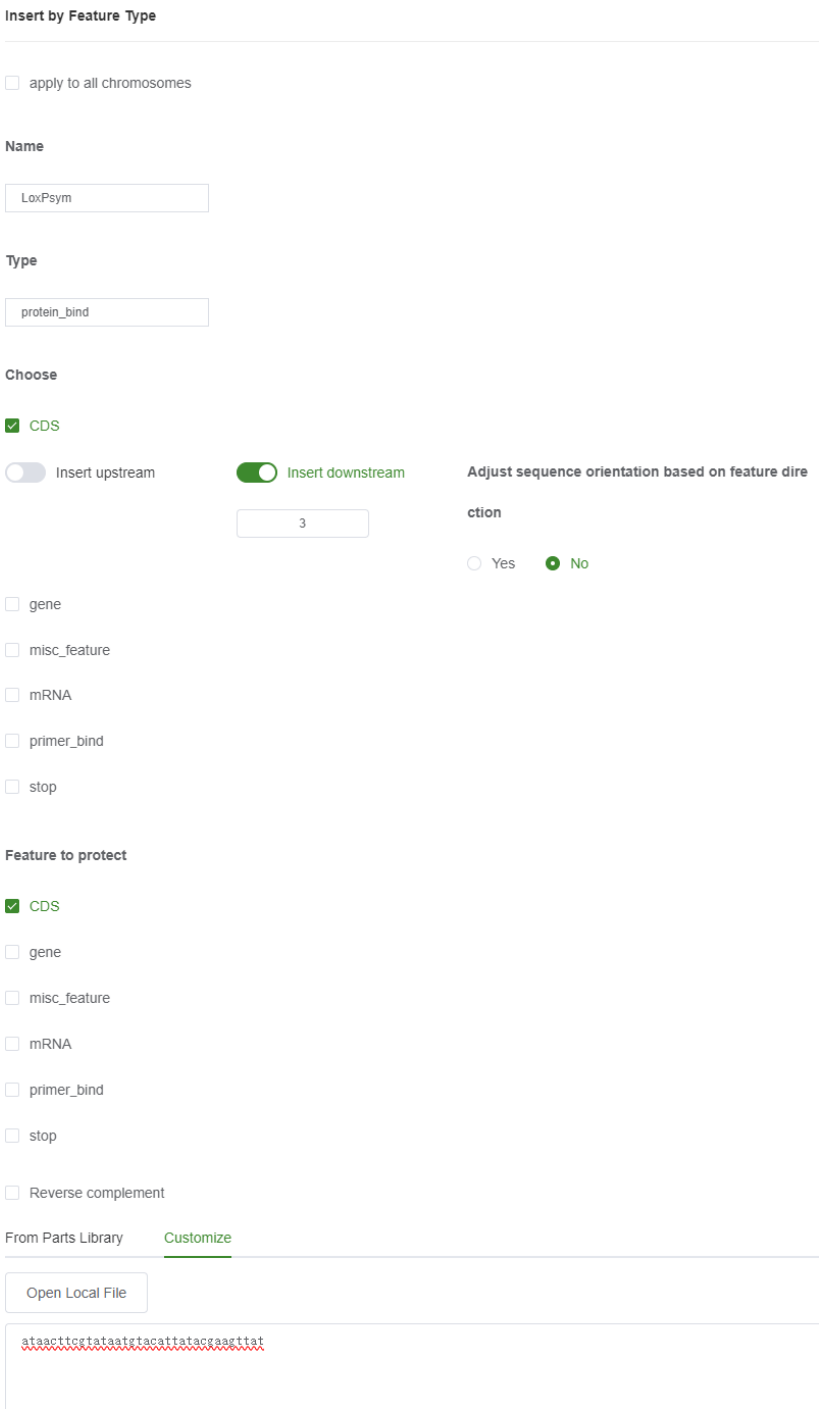

Fig. 11: Arguments of “Insert by Feature Type” for introducing SCRaMbLE system.

4 Downloading and Verification

After modification of the genome, you can go to the “Design Tool” page and find your design. For synthetic Chr16L design, at this step, the genome design is finished and ready for downloading (Fig. 12). Clicking “Download” can allow you to pack and download the FASTA, Genbank, and history

record files (Fig. 13). The resulting Genbank file should be the same as “SynMoss.Chr16L.gb” provided on Github (https://github.com/WenfeiY/P.patens_geno_design).

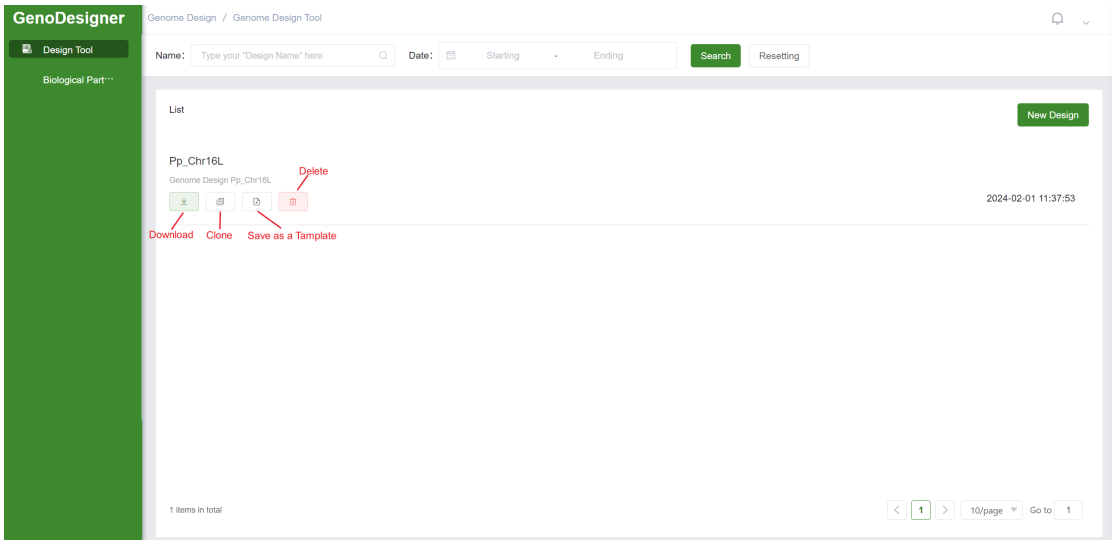

Fig. 12: Design list.

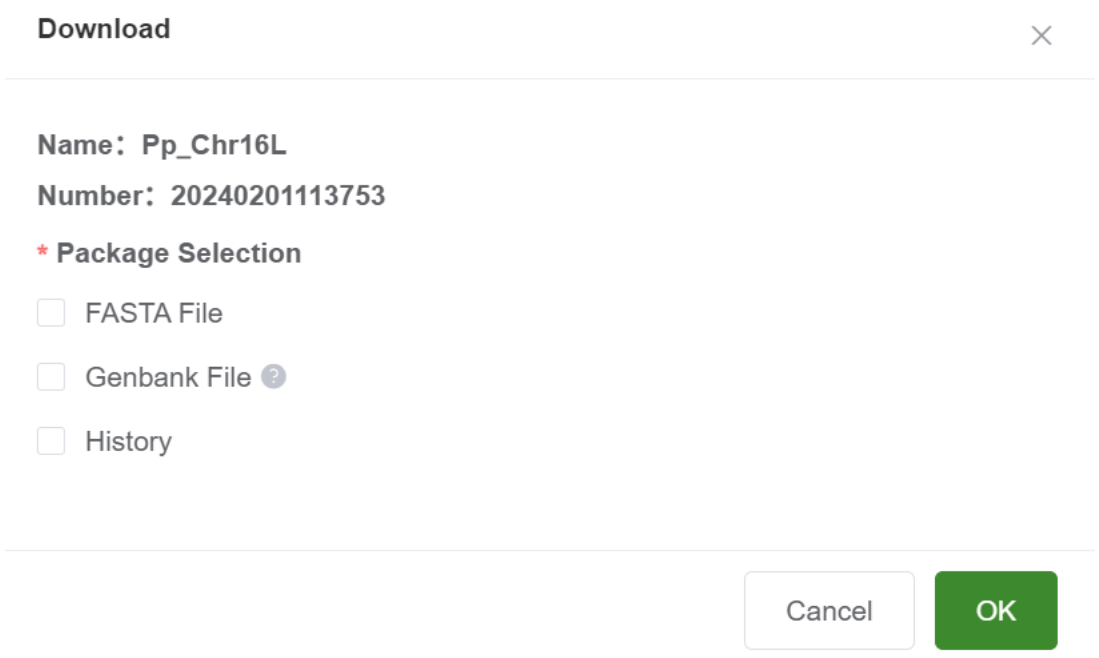

Fig. 13: Download design.