

PlasMapper 3.0—a web server for generating, editing, annotating and visualizing publication quality plasmid maps

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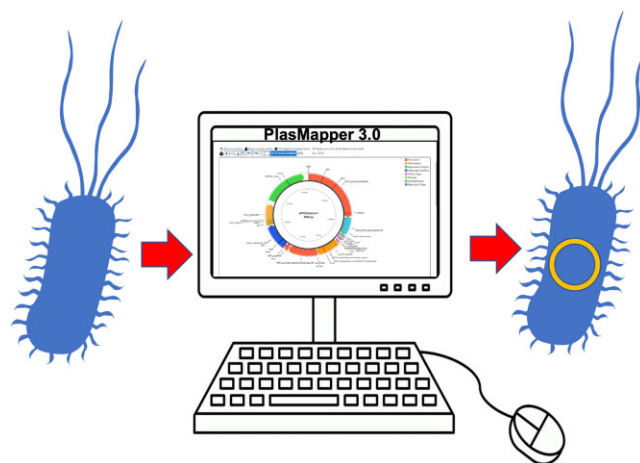
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ABSTRACT

PlasMapper 3.0 is a web server that allows users to generate, edit, annotate and interactively visualize publication quality plasmid maps. Plasmid maps are used to plan, design, share and publish critical information about gene cloning experiments. PlasMapper 3.0 is the successor to PlasMapper 2.0 and offers many features found only in commercial plasmid mapping/editing packages. PlasMapper 3.0 allows users to paste or upload plasmid sequences as input or to upload existing plasmid maps from its large database of >2000 pre-annotated plasmids (PlasMapDB). This database can be searched by plasmid names, sequence features, restriction sites, preferred host organisms, and sequence length. PlasMapper 3.0 also supports the annotation of new or never-before-seen plasmids using its own feature database that contains common promoters, terminators, regulatory sequences, replication origins, selectable markers and other features found in most cloning plasmids. PlasMapper 3.0 has several interactive sequence editors/viewers that allow users to select and view plasmid regions, insert genes, modify restriction sites or perform codon optimization. The graphics for PlasMapper 3.0 have also been substantially upgraded. It now offers an interactive, full-color plasmid viewer/editor that allows users to zoom, rotate, re-color, linearize, circularize, edit annotated features and modify plasmid images or labels to improve the esthetic qualities of their plas-

mid map and textual displays. All the plasmid images and textual displays are downloadable in multiple formats. PlasMapper 3.0 is available online at <https://plasmapper.ca>.

GRAPHICAL ABSTRACT



INTRODUCTION

The generation and editing of plasmid maps is one of the most frequently performed operations in all bioinformatics. Nearly every practicing molecular biologist has probably generated or edited a plasmid map (or cloning vector map) to guide them through a cloning or plasmid manipulation experiment (1). Because of the size and complexity of plasmid molecules, it has long been essential

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to use specialized computer programs to help identify, locate, edit and analyze the plasmid sequence as well as specialized programs to generate and visualize the resulting maps (2). The majority of modern plasmid editing/mapping software tools are either stand-alone programs or part of larger commercial, downloadable, platform-specific software packages. Examples of commercial, stand-alone plasmid mapping/editing programs include SimVector (available at <https://www.premierbiosoft.com>), SnapGene Viewer (SnapGene®), available at www.snapgene.com), SeqBuilder Pro (available at <https://www.dnastar.com/>) and Vector NTI (Invitrogen) (3). These programs support a wide variety of plasmid display options, plasmid annotation tools and sophisticated plasmid editing function. There are also several freely available downloadable programs for plasmid editing/viewing including ApE (4), which operates on Windows, MacOS and Linux, pDRAW32 (www.acaclone.com), which is Windows 10 compatible and Serial Cloner (http://serialbasics.free.fr/Serial_Cloner.html), which is compatible with MacOSX, Windows and Linux.

The requirement for downloadable programs to support plasmid mapping and editing appears to be mostly historical, if not inertial. Web technologies and web-based visualization tools have made web-enabled plasmid mappers and web-enabled plasmid editors feasible for quite some time. One example of a web-enabled plasmid mapping/editing tool is Restriction Free (RF) Cloning or RF-Cloning, which was published in 2012 (5). This easy-to-use website allows users to paste in a plasmid sequence or select from about 40 popular RF plasmids, to choose an insert sequence and display a colorful, annotated plasmid map. This website has two sequence editors (one for the plasmid and one for the inserted gene) that supports customization and reformatting of the sequence display. However, RF-cloning is a very specialized web server designed to support RF cloning and the manipulation of RF-cloning plasmids only. RF cloning is a PCR-based method that allows the insertion of essentially any sequence into any plasmid at any location without the need for restriction enzymes or ligation enzymes. While very convenient, this is not the way most cloning experiments are done. Furthermore, the RF-cloning website is somewhat limited in its offerings as users cannot manipulate, re-color or re-render the plasmid map images or modify the sequence text in any substantive manner.

Another plasmid annotation tool is pLannotate (6). This web server allows users to quickly and comprehensively annotate plasmid features using large databases of genetic parts and proteins. It also displays a graphical map of the annotated plasmid, explains the provenance of each feature annotation, and allows results to be downloaded in a variety of formats. However, pLannotate is intended primarily for annotating naturally occurring plasmids and not really built for annotating cloning vectors. Nor is it built for designing a cloning experiment, editing plasmid sequences or rendering plasmid maps or text display images with the typical annotations needed for gene or protein cloning publications or conference posters.

A more general and somewhat more capable plasmid mapping/editing web server is PlasMapper (7). The last version of PlasMapper (version 2.0) was described in 2004. This web server uses a variety of Java programs (such

as CGView (8), several components of BioJava (<http://www.biojava.org/>) along with several specialized internal databases to help it automatically annotate cloning vectors and display their circular plasmid maps. PlasMapper 2.0 also supports a wide range of textual and visual display options that allow users to easily customize the image or textual output. However, its dependency on Java and other 'dated' web technologies means that PlasMapper 2.0 is not very responsive or interactive. This means users must constantly refresh their screen to update any edits. Likewise, many of the plasmid sequences and plasmid features listed in PlasMapper 2.0 are no longer popular, supported, or even available.

Given the limitations of RF-Cloning and pLannotate as well as the dated status of PlasMapper 2.0, we decided to revisit web-based plasmid mapping and editing. In particular, we wanted to determine if recent advances in JavaScript technologies and other interactive visual display tools developed for the web could be used to implement many of the high-end interactive features found in commercial plasmid mapping/editing programs. We also wanted to investigate what tools and resources could be updated or added to the original PlasMapper to make it more appealing to today's molecular biologists.

Here we describe PlasMapper 3.0, the successor to PlasMapper 2.0. PlasMapper 3.0 is a far more interactive, visually appealing plasmid editing/mapping tool than its predecessor. PlasMapper 3.0 offers many features found only in expensive plasmid editing/mapping packages. For instance, it allows users to paste or upload any plasmid sequence as input or to upload an existing plasmid map from a custom database of >2000 pre-annotated plasmids (PlasMapDB). This database can be searched by any Boolean combination of plasmid names, sequence features, restriction sites, preferred host organism(s), and/or sequence length. PlasMapper 3.0 also supports the annotation of new or never-before-seen plasmids using its own feature database (called FeatureDB) covering common promoters, terminators, regulatory sequences, replication origins, selectable markers, and other features found in most cloning plasmids and vectors. PlasMapper 3.0 has several interactive sequence editors that allow users to select and view plasmid regions, insert genes, modify restriction sites or perform codon optimization. The graphical display functions for PlasMapper 3.0 have also been substantially upgraded over what was in PlasMapper 2.0. In particular, PlasMapper 3.0 now offers a highly interactive, colorful plasmid viewer/editor that allows users to zoom, rotate, re-color, linearize, circularize, customize annotated features and modify the plasmid images or labels to improve the aesthetic qualities of their plasmid map. PlasMapper 3.0 also allows all its colorful maps and textual displays to be downloaded in multiple formats. These and other improvements are described in more detail below.

A brief tour of PlasMapper 3.0

PlasMapper 3.0 has been completely rewritten and redesigned from the bottom to the top so that it is simpler, more flexible and easier to use than its predecessor. A screenshot of the PlasMapper 3.0 homepage is

shown in Figure 1a. The dark blue menu bar at the top of the page shows six menu options ‘Home’, ‘Browse’, ‘Help’, ‘About’, ‘Request’ and ‘Contact Us’. ‘Browse’ allows users to browse, select and upload popular, pre-annotated plasmids from PlasMapDB into the PlasMapper Editor. ‘Help’ provides a detailed illustrated tutorial of how to use PlasMapper, ‘About’ provides a brief history of PlasMapper, citation information, the web server’s general architecture, the key programs used and links to the source code. The ‘Request’ link allows users to request the addition of plasmids to PlasMapDB, features to FeatureDB (PlasMapper’s feature database) or restriction enzymes to ReDB (PlasMapper’s restriction enzyme database). The ‘Contact Us’ link allow users to contact the PlasMapper programming team for questions. The ‘Home’ page is the default display that allows users to paste or upload a plasmid file/DNA sequence into the web server. As seen in the center of the home page screen, users are presented with a pull-down menu with three options: (i) paste plasmid nucleotide sequence; (ii) upload plasmid nucleotide sequence file or (iii) select a plasmid from PlasMapDB. The default is the paste option, which allows users to paste a plasmid DNA sequence (raw or FASTA) into the text box. Clicking the purple ANNOTATE button on the lower part of the window will launch the PlasMapper Editor and display both the annotated plasmid sequence and the corresponding plasmid map. If a user chooses, instead, to upload a plasmid file (option #2), a ‘Drag-and-Drop’ box appears in the center of the screen which allows users to drag a *.txt or *.fasta file into the box whereupon the file is loaded into the web server. Clicking the purple ANNOTATE button will launch the PlasMapper Editor and display both the annotated plasmid sequence and the corresponding plasmid map. If a DNA sequence is pasted or uploaded and the ANNOTATE button is clicked, PlasMapper will run BLASTN on the plasmid sequence against its database of 471 known or common plasmid features (FeatureDB) and its database of ~250 common restriction enzymes (ReDB). The results will be displayed on the data in the PlasMapper Editor.

If a user selects the third option (Select a plasmid from PlasMapDB), a new window appears which displays the content of the PlasMapDB, which is PlasMapper’s custom database of popular, pre-annotated plasmids (Figure 1B). This database is shown as a table with seven column headings including plasmid popularity (based on the frequency with which it is accessed via PlasMapper), the plasmid parent, the host organism compatibility, plasmid features, full plasmid name, the plasmid length (in number of bases) and the supplier. Each of the columns can be sorted alphabetically or by size. The full list of 2082 plasmids in PlasMapDB can also be filtered or shortened according to a variety of selection criteria. On the left side of the PlasMapDB table is the database’s ‘filter selector’ where users can limit the size of the displayed table by placing restrictions on the name (or portion thereof) and Boolean (using the ‘and/or’ slider) combinations of listed sequence features, restriction sites, expression system, minimum sequence length or maximum sequence length. Pull-down options are available for selecting sequence features, restriction sites, and expression system (all displayed in alphabetical order). Filtering is done

instantly and the resulting filtered PlasMapDB table will display the list of plasmids meeting the selection criteria along with the number of plasmids (listed at the bottom) that match those filter criteria. Clicking on the plasmid name in the PlasMapDB table produces a pop-up window on the right side of the window that shows a thumbnail color image of the plasmid and the sequence. Clicking on the ‘Open in Editor’ hyperlink will launch the PlasMapper Editor with the selected PlasMapDB plasmid.

The PlasMapper Editor allows users to view, edit and manipulate plasmid sequences and plasmid map images. It appears as a scrolled window (rather than a new window) below the home page once the ANNOTATE button is pressed. This allows users to easily scroll back up to the home page to reload other plasmid data. As shown in Figure 2A and B, the PlasMapper Editor consists of four panels. A *plasmid map panel* (upper right), a *map editing panel* (upper left), a *plasmid sequence display panel* (lower right) and a *plasmid sequence editing panel* (lower left).

The *plasmid map panel* uses CGView.js (a JavaScript version of CGView (9)) to allow the interactive visual display of plasmid maps. The plasmid map display adheres to most conventions found for commercial plasmid viewers with genes, promoters, tags, restriction sites and other features marked in colored arrows and text labels radiating out from the circular plasmid plot. A color legend indicates what color corresponds to which plasmid feature. The name of the plasmid and its size is indicated on the inside of the plot and a ‘clock’ with the base positions is placed inside the plasmid image. Above the plasmid image frame are instructions for manipulating the plasmid image. When the zooming ‘lock button’ is unlocked (or if a colored plasmid feature has been clicked), users may zoom in and out using the scroll wheel on their mouse or trackpad on their computer. Zooming all the way in allows the plasmid DNA sequence (both forward and reverse strands) to be seen. Clicking and dragging on the plasmid map with a mouse or trackpad allows the vertical or horizontal position of the plasmid image to change. Clicking on specific color blocks on the legend allows legend colors to be changed or edited.

The *plasmid map panel* has an image control panel located immediately above the image frame with several control buttons. This includes, moving left to right, a scrolling ‘lock button’ (always locked by default) which limits zooming functions when scrolling up and down through the PlasMapper Editor. Pressing the ‘lock button’ or clicking anywhere on the colored plasmid features will lock/unlock the plasmid zooming functions. Continuing across the control panel, the ‘+’ button can be clicked to zoom in, the ‘-’ button can be clicked to zoom out, the down arrow allows the image to be downloaded, the ‘o/-’ button can be used to toggle between circular and linear views, the ‘counterclockwise arrow’ button allows the plasmid image to be rotated counterclockwise while the ‘clockwise arrow’ button allows the plasmid image to be rotate clockwise. Labels can be toggled off and on with the circular ‘label’ button and the image can be re-centered by pressing the corner frame or re-centering button. Users can select which base in the sequence they wish to see by typing in the number in the grey box to the right of the re-center button and pressing the orange ‘GO TO BASE NUMBER’ button.

A

Welcome to PlasMapper 3.0!
 PlasMapper 3.0 is a web server that allows users to generate, edit, annotate and interactively visualize publication quality plasmid maps. Plasmid maps are used ubiquitously in molecular biology and they provide a simple, visual approach to plan, design, share and publish critical information about cloning experiments. PlasMapper 3.0 has provided access to new and extended plasmid databases from which users can pick a wide variety of plasmid. Additionally, it offers an option of automated codon optimization and BLAST sequence alignment. PlasMapper 3.0 supports a variety of input formats including raw sequence, FASTA files and its own PlasMapper JSON format.

Please cite:
 1. PlasMapper 3.0:
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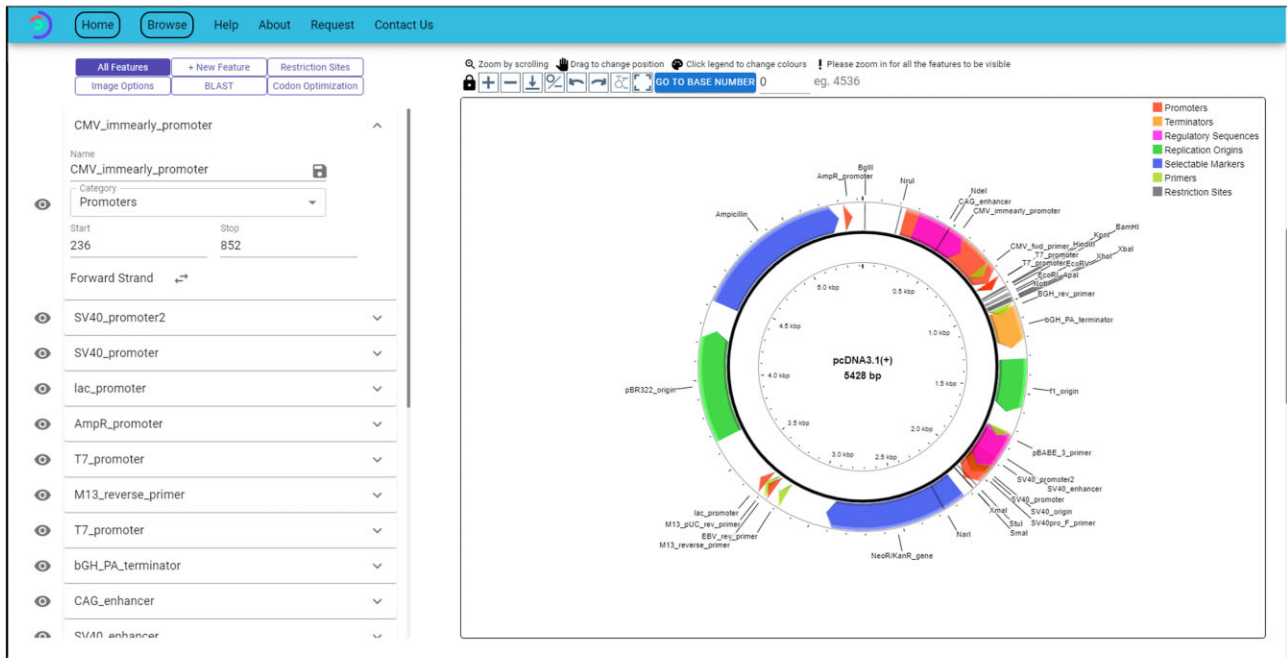
B

Results:

| Popularity ↓ | Plasmid Parent | Host Organism Compatibility | Features | Full Name | Length | Supplier |
|--------------|----------------------|----------------------------------|--|--------------------------------|--------|------------|
| 16 | pT81Luc | Bacterial Expression, Luciferase | AmpR_promoter,SV40_polyadenylation_signal,SV40_pola... | NFAT/AP-1 3x luciferase | 6378 | AddGene |
| 9 | pET28a | Bacterial Expression | T7_transcription_termination_region,lacO,pBR322_orig... | BirA in pET28a (w400-2) | 6210 | AddGene |
| 5 | pET15b | Bacterial Expression | AmpR_promoter,lacI,mouse_protein_kinase_A(PKA)gene,la... | pET15b PKA Cat | 7383 | AddGene |
| 5 | pBluescript SK II... | Bacterial Expression | lac_promoter,AmpR_promoter,pBR322_origin,f1_origin,Am... | pBS aP2 promoter (5.4kb) polyA | 8734 | AddGene |
| 4 | pGADT7 | Yeast Expression | S_cerevisiae_ADH1_promoter,lac_promoter,AmpR_promot... | pGADT7-GW | 9638 | AddGene |
| 4 | pcDNA3.1-ID01... | Mammalian Expression | CMV IMMearly_promoter,SV40_promoter2,SV40_promoter... | pcDNA3.1-ID01-p2a-eGFP | 7373 | GeneScript |
| 3 | pET16b | Bacterial Expression | AmpR_promoter,T7_transcription_termination_region,lacI... | pET16b.Pfu | 8032 | AddGene |
| 2 | pJED103 | Yeast Expression, CRISPR | lac_promoter,AmpR_promoter,yADH1_terminator,pBR322... | pTDH3-dCas9-Mxi1 | 11205 | AddGene |
| 2 | pGreenII | Plant Expression | lac_promoter,cauliflower_mosaic_virus_35S_terminator,pB... | pGreenII DR5v2-ntdTomato/... | 9680 | AddGene |
| 2 | pBS SKplus | Bacterial Expression | lac_promoter,AmpR_promoter,pBR322_origin,f1_origin,Am... | pBS hShh (CT401) | 4544 | AddGene |

Figure 1. Screenshots of the PlasMapper 3.0 homepage and the PlasMapDB database. (A) The menu shown across the top of the homepage provides various navigation options including: 'Home', 'Browse', 'Help', 'About', 'Request' and 'Contact Us'. (B) A screenshot showing the content of PlasMapDB and the selection of available plasmids.

A



B

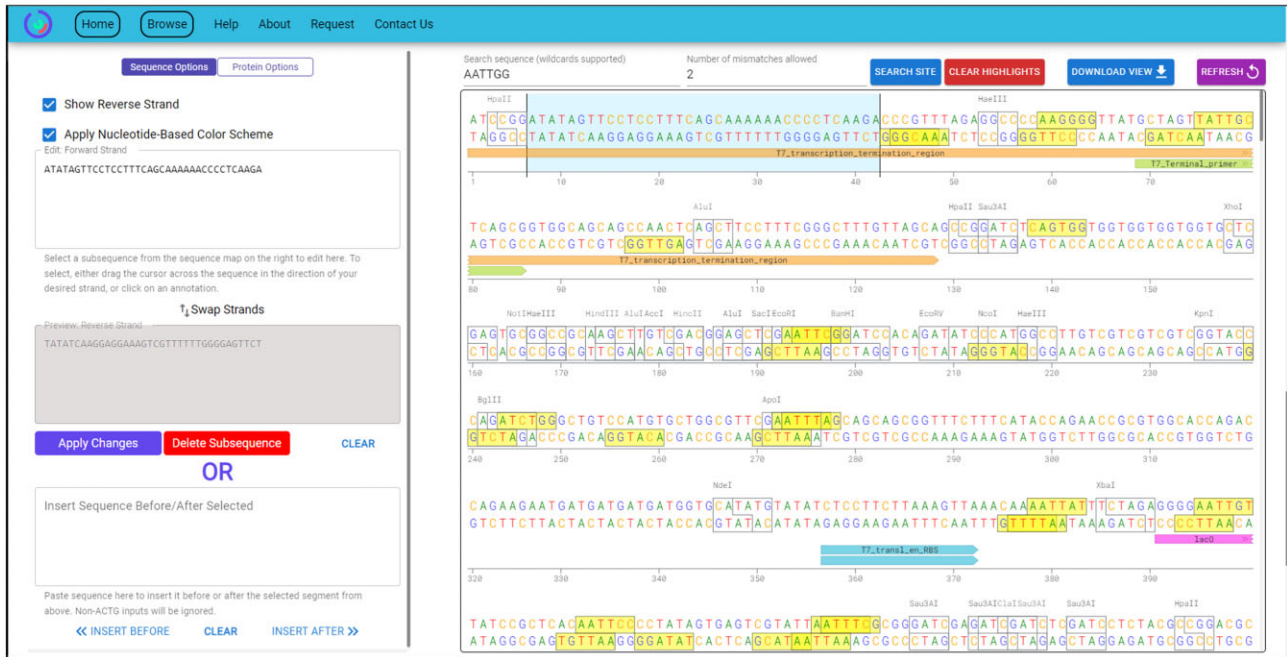


Figure 2. Screenshots of the PlasMapper Editor. (A) A screenshot showing details of the plasmid map panel (upper right) and the plasmid map editing panel (upper left). (B) A screenshot showing details of the plasmid sequence display panel (lower right) and a plasmid sequence editing panel (lower left).

The *map editing panel* on the left side of the PlasMapper Editor is designed to help users easily edit and modify the plasmid map and plasmid labels. As seen in Figure 2A, the *map editing panel* displays a scrollable, pull-down menu list of features, such as genes, promoters, restriction sites, etc. Pressing on the named feature pull-down provides details on the feature name, its general feature category and its position in the plasmid (base number and strand). The feature name can be edited and saved (by clicking on the small floppy disk icon) to allow that feature to be more properly displayed on the plasmid map shown on the right panel. This is particularly useful if the feature has an unusually long, unusually short or a potentially confusing name. Individual features can be toggled on or off on the plasmid map by clicking on the ‘eye’ icons on the left of the list. Above the feature list are a number of feature control buttons. The default displays ‘All Features’. Users can add or label new features to the plasmid map by clicking the ‘+ New Feature’ button and filling out the required information. Users can also edit the number and type of restriction sites displayed on the plasmid map by clicking on the ‘Restriction Sites’ button and then checking (or unchecking) different check boxes associated with restriction site names. This allows the quick addition or removal of multiple restriction sites. The ‘Image Options’ button allows users to change the plasmid map layout, add GC content data, toggle off/on color, change the plasmid title and download the map image in different formats (PNG, SVG or JSON [JavaScript Object Notation] object). Users can also toggle the ‘poster view’ option which generates a simplified version of the plasmid map which can be used for publications or posters. Users can also upload a previously saved PlasMapper JSON file to continue editing it. The ‘BLAST’ button allows users to run the plasmid sequence against the NCBI nucleotide database. Users must provide their email address to have the BLAST results properly delivered. The ‘Codon Optimization’ button allows users to optimize the codons of a selected gene (usually their gene of interest) for expression in a specific host organism. To perform codon optimization, users must select the start and end position of their gene of interest. This can be done by typing in the positions or by clicking on the gene/feature of interest on the plasmid map to the right. This feature has been implemented using the tool Optipyzer (<https://github.com/nleroy917/optipyzer>). Once that information is provided, users must select the host organism from the pull-down menu and then press the ‘Apply Optimization’ button. The optimized DNA sequence is then generated. The *plasmid sequence display panel* and *plasmid sequence editing panel* (see Figure 2B) allow users to manipulate and view the plasmid at the sequence level. The plasmid sequence display panel was built using the JavaScript library SeqViz (<https://github.com/Lattice-Automation/seqviz>), a React-based sequence visualization package available via the JavaScript Node Package Manager. (<https://www.npmjs.com/>). SeqViz is an open-source tool that was built to be a customizable library that users can plug into an existing online resource. It provides users with a colorful, easy-to-use viewer for manipulating genomic sequences. This sequence display tool allows the full plasmid sequence to be displayed and scrolled through in a manner similar to most standard sequence ed-

itors. The default display shows 80 bases per line (forward and reverse strand) and it uses different colors to display different bases (A, T, G and C) along with a numbered sequence ruler (below the sequence) to facilitate base numbering or base location. Restriction sites and other plasmid features are marked and colored identically in this sequence display as with the plasmid map (above). The *plasmid sequence display panel* and *plasmid sequence editing panel* are closely coupled. Users can click on a position between bases in the *plasmid sequence display panel* and a vertical line will appear. Users can then enter a gene (or other feature) sequence into the ‘Insert Sequence’ text box on the left *plasmid sequence editing panel*. Alternately, users can select a region of the sequence by clicking and dragging over a sequence region in the *plasmid sequence display panel* and a light blue highlight band will appear over the selected sequence region. Users can also highlight a region on the sequence by dragging the line across the sequence map and the direction in which the user will drag the vertical line will determine the selected strand which can be swapped later by using the ‘Swap Strands’ button in the *plasmid sequence editing panel*. Users can either modify the sequence in the ‘Edit Sequence’ textbox on the left *plasmid sequence editing panel* or they can enter a gene (or other feature) sequence into the ‘Insert Sequence’ textbox on the left *plasmid sequence editing panel*. Users have the option of applying changes, deleting the selected subsequence, clearing the operation or performing codon optimizations on the selected region by clicking the corresponding buttons.

The *plasmid sequence editing panel* on the left side of the screen has two buttons. One is called ‘DNA Sequence Options’ and the other is called ‘Protein Sequence Options’. When the ‘DNA Sequence Options’ button is selected, users can turn off/on the reverse strand, change the number of displayed bases per line (60, 80, 100), turn the base coloring on or off, hide or display sequence features, or download the current sequence view as a PNG image. This function also allows users to perform the sequence edits and insertions described earlier. The ‘Protein Sequence Options’ allows users to display protein sequences (for selected genes) below the displayed DNA sequence. Several amino acid display options are available. Users can choose to translate: (i) only a selected region of the plasmid (by providing start/stop positions), (ii) only previously identified gene coding features or (iii) all possible ORFs in the plasmid. Users can also change the size of the displayed amino acid sequence (full size/half size characters), switch between a single letter or three letter code, toggle the amino acid coloring on or off or download the current sequence view as a PNG image.

Additional details concerning the operation of PlasMapper 3.0 and other options not mentioned here can be found under the ‘Help’ menu at the top of the PlasMapper home page. This tutorial provides a richly illustrated set of instructions with plenty of examples on how to use PlasMapper to its fullest.

PlasMapper’s databases

A key feature of PlasMapper 3.0 is its extensive collection of databases designed specifically for annotating plasmids and cloning vectors. There are three key databases

Table 1. A list of the sequence feature types found in FeatureDB

| Type of feature | Number of features |
|-----------------------|--------------------|
| Promoter | 50 |
| Terminator | 29 |
| Regulatory sequence | 65 |
| Replication origin | 12 |
| Selectable marker | 26 |
| Reporter gene | 40 |
| Two-hybrid gene | 12 |
| Localization sequence | 15 |
| Affinity tag | 21 |
| Gene | 7 |
| Primer | 77 |
| Miscellaneous | 117 |
| Total | 471 |

in PlasMapper 3.0: (i) PlasMapDB (PlasMapper's pre-annotated plasmid database); (ii) FeatureDB (PlasMapper's feature database) and (iii) ReDB (PlasMapper's restriction enzyme database). PlasMapDB consists of 2082 pre-annotated, commonly used cloning plasmids covering those vectors that can be used for bacterial, yeast, insect cell, plant/plant cell and mammalian cell cloning. PlasMapper's plasmid collection was assembled from AddGene's freely available collection of plasmids (<https://www.addgene.org/>), commercial plasmids from GeneScript (www.genescript.com), plasmids from RF-cloning (5) and other popular plasmids from a number of miscellaneous sources. Each entry in PlasMapDB consists of two JSON files, with one containing plasmid DNA sequences and the other containing plasmid metadata. The metadata includes the plasmid name, the sequence length, the plasmid 'parent', the supplier, the preferred host, the level of popularity and the identified features. The features (types and positions) were identified by conducting BLASTN (10) searches against FeatureDB for each plasmid in PlasMapDB. PlasMapDB was partitioned into two separate files to enable a number of performance improvements (described later).

FeatureDB consists of a single FASTA file with 471 carefully selected and annotated plasmid features broken down into 12 feature categories. These are shown in Table 1. FeatureDB was created by combining the original FASTA formatted PlasMapper 2.0 feature database (which had 336 features) with AddGene's sequence feature database (<https://www.addgene.org/>) followed by extensive manual editing, duplicate removal, validating and cleaning.

ReDB consists of the names, restriction site sequences and cutting sites for 250 of the most popular, commercially available restriction enzymes used in molecular biology. While hundreds of restriction enzymes are known ((11), Promega Corporation (<https://www.promega.com>)), not all are commercially available and not all are ideal for cloning or plasmid manipulation. Therefore, it was decided to keep this collection relatively small for PlasMapper 3.0.

Molecular biology techniques, along with plasmid designs are continuously evolving. To support this evolution, PlasMapper 3.0 has introduced a 'Request' option located at the top of the home page menu bar. The 'Request' link allows users to request the addition of plasmids to PlasMapDB, features to FeatureDB or restriction enzymes

to ReDB. Users must complete a request form by selecting one of the three options (Add Plasmid, Add Feature, Add Restriction Enzyme) from the pull-down menu. To add a plasmid, users must provide the full plasmid sequence (without any cloned target gene), the plasmid name, the plasmid parent or plasmid type, the preferred host organism (chosen from a pull-down menu), the supplier, a short rationale and the submitter's email. Once all information is provided, users can submit the request by pressing the purple 'Submit' button. The plasmid will be auto-annotated via PlasMapper's annotation pipeline, checked for redundancy against the existing PlasMapDB list and manually evaluated by the PlasMapper curators. If the requested plasmid passes all evaluations, it will be added to PlasMapDB and an email notification will be sent to the submitter. Similar submission and validation mechanisms are provided for users to request new features (promoters, antibiotic resistance genes, terminators, tags, etc.) and new restriction enzymes. Every effort will be made to consider and add all legitimate requests. Similarly, if users notice errors or inconsistencies in plasmid annotations, features or feature annotations, they are encouraged to notify the PlasMapper team through the 'Contact Us' link at the top of the home page menu bar.

PlasMapper 3.0 implementation

As noted earlier, PlasMapper 3.0 represents a complete redesign and a total rewrite of PlasMapper 2.0. PlasMapper 2.0 was originally written as a single monolithic Java program with the front end tightly connected to the back-end. This made updates difficult and bug fixes challenging. PlasMapper 3.0 was designed to have a clearly delineated frontend (built using Gatsby React, JavaScript and HTML) and backend (built using Django REST and Python). The frontend uses Gatsby14, a React-based frontend framework known for its speed and responsiveness. Several other external JavaScript libraries are used, including CGView.js (9) for plasmid rendering, SeqViz for sequence viewing and Material UI for managing the user interface. The backend uses the Django REST Framework for the application programming interface (API) that provides annotation functionality and access to PlasMapDB to the frontend through HTTP requests.

PlasMapper 3.0 consists of a single instance where the backend is deployed using Nginx and Gunicorn, and the frontend is deployed using Nginx. By moving away from the monolithic architecture used in PlasMapper 2.0 towards a separate API and a separate frontend, interaction between the backend and frontend could be limited to RESTful HTTP requests. This setup allows for much easier software maintenance. Furthermore, any future expansion of functionality can simply be added as a set of API endpoints and new pages in the frontend that will call those endpoints using AJAX (Asynchronous Javascript and XML (or JSON)). Furthermore, the RESTful design of the API allows the PlasMapper databases to be stateless. This means that the frontend and backend need not concern themselves with the handling state of interaction, greatly simplifying development and maintenance.

Table 2. A comparison of select plasmid editing features with PlasMapper 3.0 and several other plasmid mapping/editing tools

| | PlasMapper 3.0 | PlasMapper 2.0 | SnapGene viewer | SeqBuilder Pro | Serial cloner | Restriction free cloning | ApE |
|------------------------------|----------------|----------------|-----------------|----------------|---------------|--------------------------|--------------|
| Availability | Website | Website | Downloadable | Downloadable | Downloadable | Website | Downloadable |
| Pricing | Free | Free | Paid | Paid | Free | Free | Free |
| Auto annotation | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Searchable plasmid database | ✓ | × | ✓ | * | * | × | × |
| Find restriction sites | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Find ORFs | ✓ | ✓ | ✓ | ✓ | ✓ | × | ✓ |
| Custom features | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Save edits for later | ✓ | × | ✓ | ✓ | × | × | ✓ |
| Interactive sequence editing | ✓ | × | ✓ | ✓ | × | × | ✓ |
| Interactive feature editing | ✓ | × | ✓ | ✓ | ✓ | × | ✓ |
| Filter restriction enzymes | ✓ | × | ✓ | ✓ | ✓ | × | ✓ |
| Translate DNA to protein | ✓ | × | ✓ | × | ✓ | × | ✓ |
| Edit history | × | × | ✓ | ✓ | ✓ | × | ✓ |

*Has a browser in the application that directs to NCBI services.

The PlasMapper 3.0 backend consists of a set of plasmid database (PlasMapDB) and sequence feature databases (FeatureDB and ReDB) in addition to a RESTful HTTP API that performs various queries and operations against these databases. FeatureDB and ReDB files are FASTA formatted, while PlasMapDB files are JSON formatted. When a sequence search is performed, the sequence is passed to a standalone command line version of the BLAST+ suite (10). Specifically, if a new plasmid sequence is submitted for annotation, BLASTN is used to find which features from FeatureDB have significant sequence similarity with any part of the given plasmid DNA sequence. BLASTN hits are then filtered based on the same optimized criteria used in PlasMapper 2.0 (7). The output generates several lists of JSON objects representing sequence features from many different classes such as promoters, terminators, replication origins, and many other common cloning features. Each feature object contains all the information needed by the PlasMapper frontend to properly display the feature on the plasmid map. If users request or upload an existing PlasMapDB plasmid, an API request is sent, which returns a JSON file containing the DNA sequence of that plasmid along with multiple (separate) JSON objects corresponding to the plasmid metadata, which include the name, sequence length, preferred host, supplier, etc. The reason why we separated the DNA sequence file from the other metadata was to improve performance. We found that if the sequences for each plasmid were preloaded along with the other metadata, this preload operation become too expensive.

The PlasMapper 3.0 frontend, which was built using a combination of Gatsby React, JavaScript and HTML, has already been described in earlier sections of this manuscript. PlasMapper 3.0 is compatible with multiple browsers including MS-Edge, FireFox, Safari, Opera, Chrome, iOS and Android. Note that certain cascading style sheet (CSS) properties used in PlasMapper's design are not supported in several versions of Internet Explorer (IE). Therefore, we do not recommend IE for use with PlasMapper 3.0. Additionally, JavaScript must be enabled on each user's browser for the web server to work properly. The PlasMapper 3.0 web server is available at <https://plasmapper.ca>. Likewise,

the source code is available at GitHub as described in the 'About' menu.

Comparison to other plasmid editing/mapping packages

Our intent with developing PlasMapper 3.0 was not to simply update an old and outdated web server, but to make the new version more capable and more competitive with modern (commercial) plasmid mapping/editing packages. While a comprehensive comparison against all available packages (general and specific) was not possible, we have nevertheless conducted a detailed comparison between PlasMapper 3.0 and six other plasmid editing/mapping programs or web servers, including PlasMapper 2.0, SnapGeneViewer, SeqBuilder Pro, Serial Cloner, RF-Cloning and ApE. These are summarized in Table 2. As can be seen in this comparison, PlasMapper 3.0 offers many of the same features provided by high-end commercial programs and substantially more plasmid editing/mapping features than freeware programs or web servers. The one capability PlasMapper 3.0 currently lacks is an edit history, which is unfortunately not feasible with its current architecture.

CONCLUSIONS

We have described a substantially improved version of the PlasMapper plasmid mapping/editing suite of web servers, called PlasMapper 3.0. This new web server allows users to generate, edit, annotate and interactively visualize publication quality plasmid maps. PlasMapper 3.0 represents a complete redesign and a total rewrite of its predecessor (PlasMapper 2.0). These changes, which include the use of Gatsby React, JavaScript and HTML for the frontend and an improved backend design that increases speed and supports more facile updating, mean that PlasMapper 3.0 is now able to offer many features found only in commercial plasmid mapping/editing packages. For instance, PlasMapper 3.0 offers a full-color plasmid viewer/editor that allows users to interactively zoom, rotate, re-color, edit annotated features and modify the image or image labels to improve the esthetic qualities of their plasmid map. PlasMapper 3.0 also allows users to paste or upload plasmid se-

quences as input or to upload existing plasmid maps from a large database of > 2000 pre-annotated plasmids. This pre-annotated plasmid database can be filtered or searched by plasmid names, sequence features, restriction sites, preferred host organism, and sequence length. PlasMapper 3.0 also supports the annotation of new or never-before-seen plasmids using its own feature database that contains common promoters, terminators, regulatory sequences, replication origins, selectable markers and other features found in most plasmids. PlasMapper 3.0 has several interactive text editors/viewers that allow users to select and view plasmid regions, insert genes, modify restriction sites or perform codon optimization. Support for the addition of new plasmid features and new plasmids has been added, giving users greater control over the content of PlasMapper and a richly illustrated tutorial with multiple examples has been developed. However, we welcome and encourage suggestions and feedback from the molecular biology community.

We believe the improvements and additions in PlasMapper 3.0 are substantial, representing a quantum leap in the capabilities of what can be offered through web-based plasmid mapping and editing. By making PlasMapper 3.0 freely available (both as a server and a downloadable program), we hope that past limitations regarding cost, consistency or accessibility to rendering, editing, sharing and viewing plasmid maps will be greatly reduced.

DATA AVAILABILITY

PlasMapper 3.0 is available online at <https://plasmapper.ca>. The source code for PlasMapper 3.0 along with all of its accompanying databases is available for users to download, install and run locally. The link to the bitbucket page for PlasMapper 3.0 is available through the home page (under “About”).

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