MacPlasmap

Version 1.82

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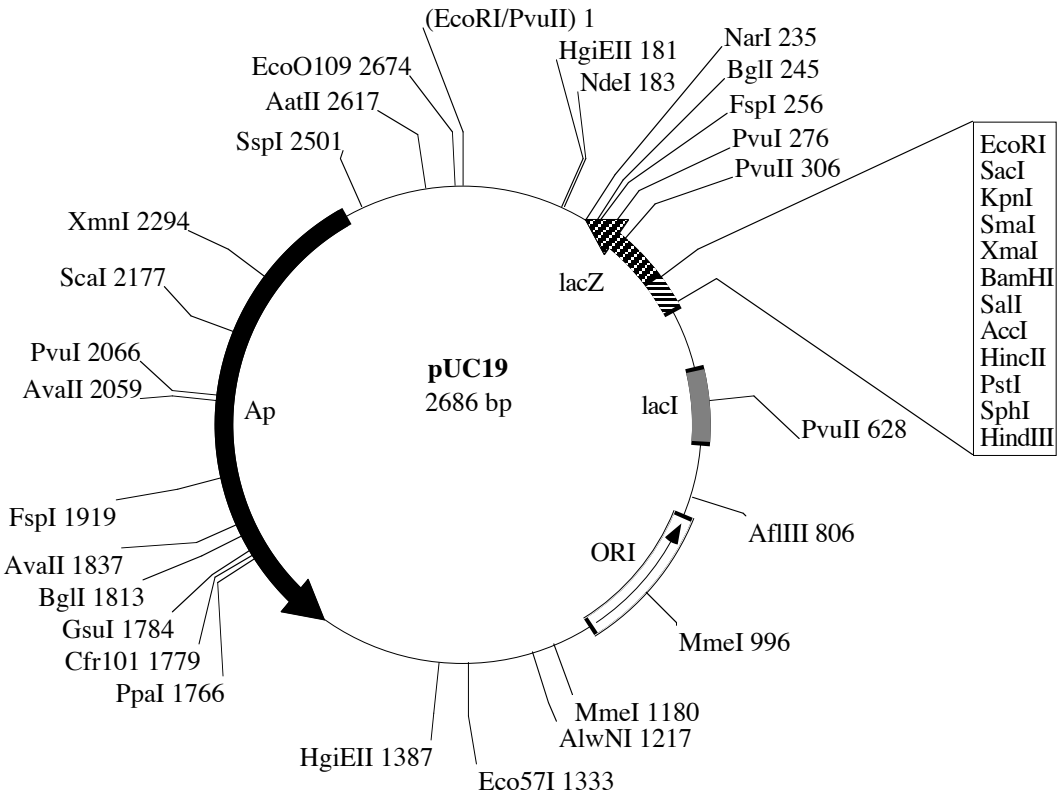

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• Note: Earlier versions of this program were distributed by Kinko's as part of their Academic Courseware Exchange package. Please treat this version as a free upgrade if you purchased MacPlasmap previously.

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INTRODUCTION

Welcome to MacPlasmap

If your study or research involves preparation of circular plasmid maps, MacPlasmap may be the tool for you. It draws, stores, and prints high-quality circular plasmid maps with the data you specify. As a molecular biologist and a Macintosh user, you have probably tried to do this on a Macintosh computer, and you may have been aware of the following inherent difficulties:

- Precise positioning of restriction sites and genes on a circle.
- Attaching an arc (representing a gene) onto a circle.
- Drawing maps of different sizes based on related, previous maps.
- Converting one type of map to a different type.

MacPlasmap is designed to overcome all of the above while still retaining other essential features of a drawing program. As a stand-alone Macintosh application, MacPlasmap supports such common facilities as data entry, saving, retrieving, and printing. If necessary, plasmid maps produced by MacPlasmap can be transferred to other advanced drawing programs for modifications. Keeping plasmid data on disk or in printed form also makes it easy to organize plasmid construction information consistently. Consequently, you can use MacPlasmap both as a plasmid data manager and a plasmid drawing aid.

About this manual

This manual is intended to provide detailed information for the efficient use of MacPlasmap. The three major sections in the rest of this manual are organized as follows:

Overview of MacPlasmap introduces basic features and some useful concepts of the program. It will be helpful if you at least scan it through.

Drawing Plasmid Maps can be used as a tutorial for drawing plasmid maps from start to finish. You will find references for individual editing functions such as entry and editing of restriction sites, genes, polylinkers, etc.

MacPlasmap References lists all menu commands for quick reference.

As a reminder, there is also a help screen under the **File** menu in MacPlasmap. You can probably get enough information from there to start using the program.

What you need to know

To use MacPlasmap and this manual, it is assumed that you are already familiar with the essential concepts of circular plasmid maps and the Macintosh computer. Experience with any typical Macintosh program should enable you to start using MacPlasmap easily. If you are new to Macintosh, you may find it helpful to familiarize yourself with basic techniques of clicking, dragging, menu selection, etc.

System Requirement

The minimum configuration required to run MacPlasmap is a Macintosh 512K with system version 4.1 or newer. The program is able to notify you about the use of older systems or potential memory shortages. Printing for Apple ImageWriter, ImageWriter II, and LaserWriters is supported. The program works well with Macintosh II and Multifinder.

Acknowledgement

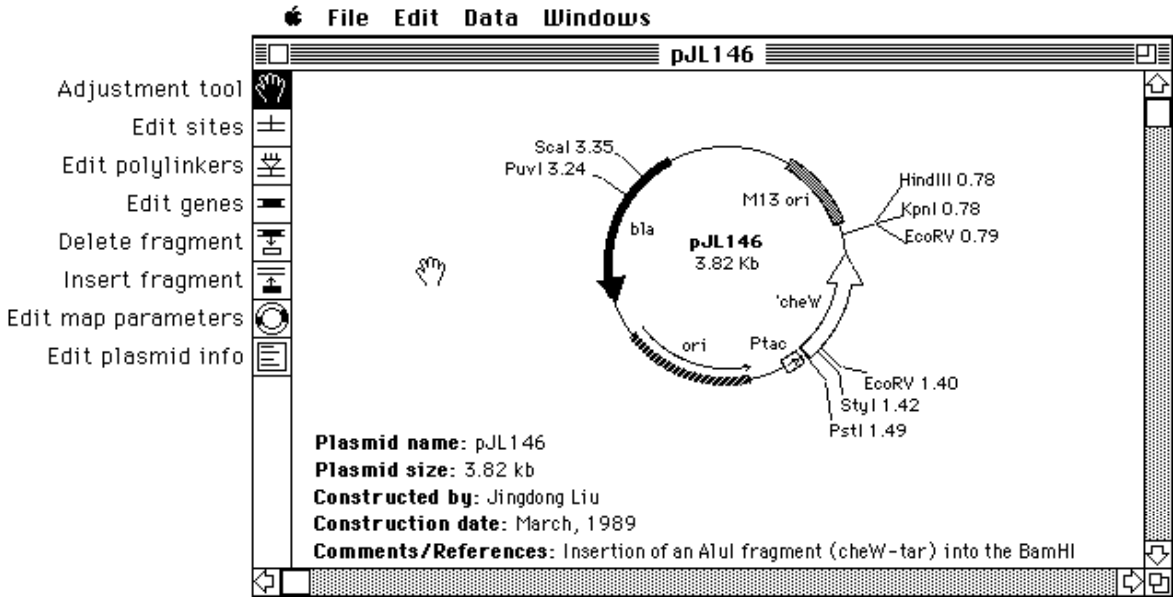
I would like to thank Dr. Sandy Parkinson for his critical comments and suggestions on the programming and design of this program. I am also grateful to many of my colleagues, particularly Tom Morrison and Jin Chen, who provided valuable feedbacks about earlier versions of this program. This program is written in Think's Lightspeed Pascal version 2.0.

OVERVIEW OF MacPlasmap

This section provides an overview of plasmid map drawing in MacPlasmap. It also introduces some useful concepts based on which MacPlasmap handles various plasmid data, and some general techniques of data entry and editing.

The MacPlasmap desktop

A plasmid map is always drawn in a window on the startup of MacPlasmap. This can be a map from the document you double-clicked on, or a pUC19 map if the program was launched by double-clicking on the application icon. From now on you can start drawing new maps, or modifying old ones. Illustrated below is MacPlasmap's desktop which you will find it worthwhile to spend a moment to get familiar with.



There are eight different icons on the left side of the window, and a plasmid map within the window. Seven of the icons constitute different editing tools, and the adjustment tool is used to adjust the positions of text labels and the map circle in a plasmid map.

Three methods of data entry and editing

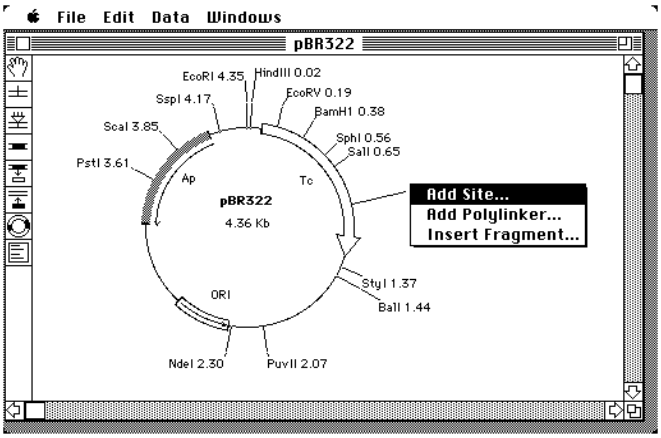
You can enter and edit plasmid data by any of the following three methods, all of which invoke the use of various dialog boxes:

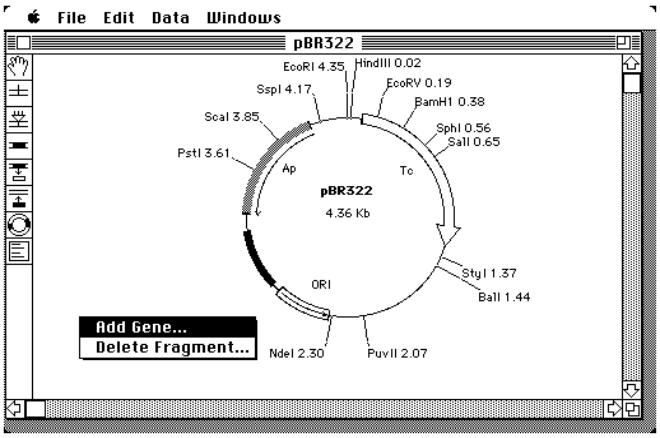
1) Choosing commands from the **Data** menu.

2) Clicking on tool icons. Clicking on a tool icon on the left side of the active window will have the same effect as choosing the corresponding command from the **Data** menu.

3) Clicking and dragging within the drawing window. You will probably find this the easiest way of entering map data. Clicking and/or dragging the mouse within the drawing window, but **outside** the plasmid map circle, will produce a flashing number indicating the position coordinate at that site of the map circle. After releasing the mouse at the position of you interest, you can start one of three operations: adding a site, adding a polylinker, or inserting a fragment. This is done by a second click within the coordinate box and then, without releasing the mouse, choosing commands from a pop-up menu. Likewise, clicking and dragging **inside** the map circle, and a subsequent click within a coordinate-containing box will invoke a pop-up menu that allows entry of a gene, or deletion of a fragment. When an editing dialog box is activated by this method, the number coordinate(s) will be automatically filled in appropriate fields of dialog boxes.

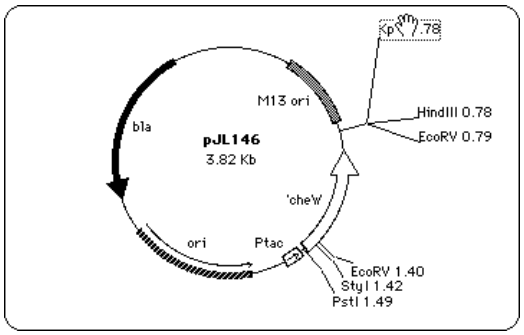
An example of popup menus for site and gene editing is shown below:



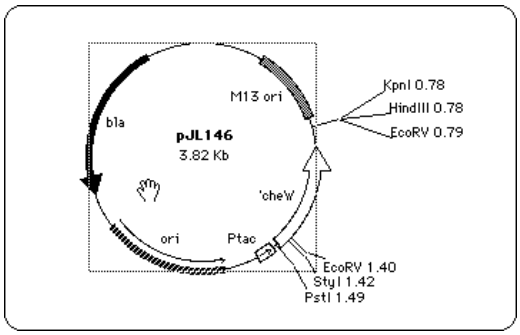


Map adjustment

When MacPlasmap draws plasmid maps, it tries its best to properly position all the text labels. However you can re-position them if they do not appear satisfactory. This is done by first selecting the adjustment tool (the hand icon) at the left side of the window and then clicking and dragging over the text you want to move. The entire plasmid map can also be moved by clicking and dragging inside the map circle (see diagram below). During this process the cursor appears as hand-shaped. You cannot enter plasmid data by the clicking and dragging method in this mode.



Adjust site



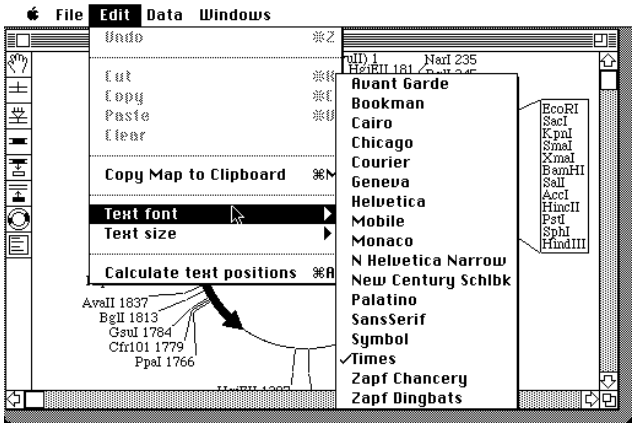
Adjust plasmid circle

Double-clicking anywhere in the window will return to the data entry and editing mode. The two modes can also be easily switched by pressing the Space key.

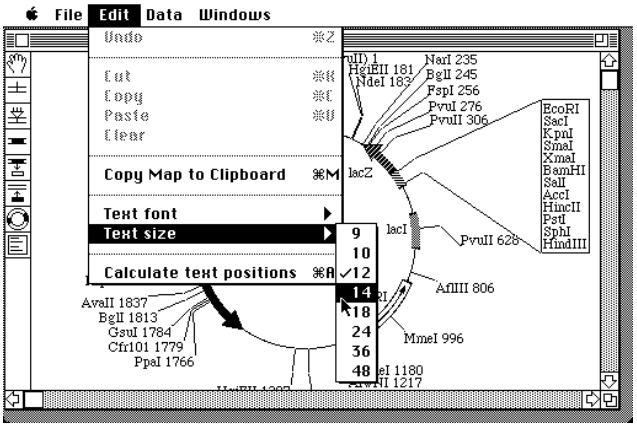
Text display

MacPlasmap provides limited control over display and printing of text items in plasmid maps. Text font and size can be chosen from two hierarchical menus under the **Edit** menu (see figure below). Current font and size are check-marked in the menus. In MacPlasmap version 1.8, each map can have only one text font and size.

> *Note* 1) The font list shown in the figure is only a sample. Your choice of fonts depends on the number of fonts available in your system software.
> 2) The default text setting is Geneva 9 points which gives clear display on the screen and ImageWriter printers. On LaserWriter printers, Times and Helvetica fonts usually give better results.



Text font



Text size

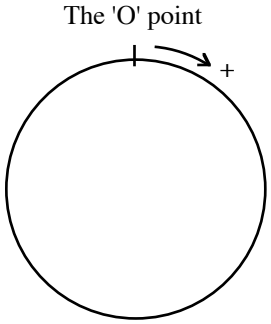
Plasmid map data used by MacPlasmap

In MacPlasmap, the following groups of data can be specified and used to control the drawing of a plasmid map:

Data Type	Information Required
Restriction sites	Site name, location, and show (or hide) site position.
Genes	Gene name, start, end, orientation, shading pattern, arrow type, and segment thickness
Polylinkers	Location and sites. Endpoint optional.
Map parameters	Map circle size, thickness, map types and display unit (kb or bp)
Plasmid info	Plasmid name, size, construction data and the author, and references/comments

The reference point

MacPlasmap draws plasmid maps by using a '0' point, located at the top of the circle, as the location reference (see figure below). Any numerical data input (for site and gene locations, for example) should use this site as the reference point. The number increases clockwise and decreases counterclockwise.



Numbering system

The basic unit for numbers related to plasmid properties can either be kilobase (kb) or basepair (bp). The kb values are automatically expressed to two decimal points. Map parameters such as circle size and thickness are expressed in screen dots (or pixels) and they must be integer values.

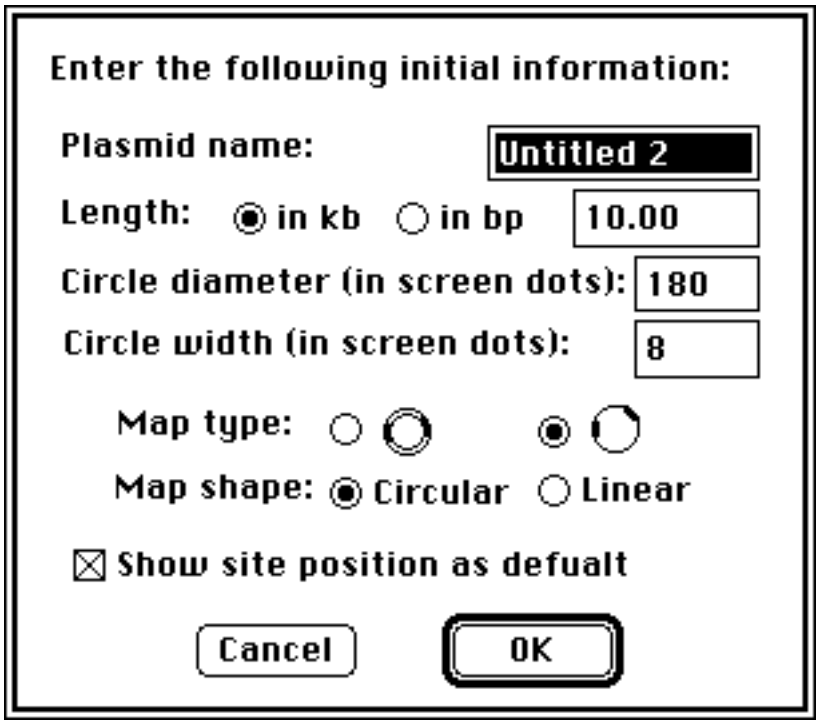
DRAWING PLASMID MAPS

This section describes typical ways of drawing plasmid maps with MacPlasmap. All the editing functions are explained in some detail. Saving and retrieving plasmid files and printing plasmid maps are also described in this section.

Starting

You can draw a new plasmid map either from scratch or from an existing plasmid map through stepwise modifications.

1)From scratch. Choose **New...** from the **File** menu. This will bring up a dialog box asking for some initial information that MacPlasmap needs to draw a new plasmid map. Fill in the necessary data, or use the default settings, which are shown in the dialog box below:



Some important points about this dialog box are discussed below:

• The size and width of the map circle are expressed in screen dots (pixels). They must be integer values. (The screen of Macintosh plus and SE is 512×342 dots, and that of Macintosh II is 640×480 dots.)

• MacPlasmap can draw two types of map shown in the **Map type** field. In the second type (single circle), the circle width attribute is used to represent the default thickness of gene segments.

• The state of the **Show site position as default** check box determines the default value of **Show site position** in the **Site editing** dialog box (see page 10). These two check boxes have the same value when a new site is initially created, but the **Show site position** box can be subsquently changed for each individual site as desired, without affecting the state of **Show site position as default**. If **Show site position** is on, then the site position for that site will be drawn on the map, otherwise only the site name will be drawn.

Note If you have used earlier versions of MacPlasmap, you will notice that **Show site position as default** is a replacement of **Number site position**, and these two buttons work differently.

• Linear map drawing is not supported in the current version of MacPlasmap.

2) From an existing plasmid map. Choose **Open...** from the **File** menu to open a plasmid data file, and change the plasmid parameters to those desired for the new plasmid.

Caution To prevent overwriting the old plasmid by modified data, it is recommended that you always change the plasmid name first (through the map parameter editing dialog box, page 16). MacPlasmap recognizes the new name as a new plasmid, and presents the **Save As...** dialog box when you choose **Save** (or Command-**S**). Alternatively, you can use **Save As...** to change the document name of the modified plasmid map.

In either of the above cases, further editing is done through the use of seven dialog boxes permitting the editing of restriction sites, genes, polylinkers, insertion and deletion of fragments, references and comments, and overall appearance of the map.

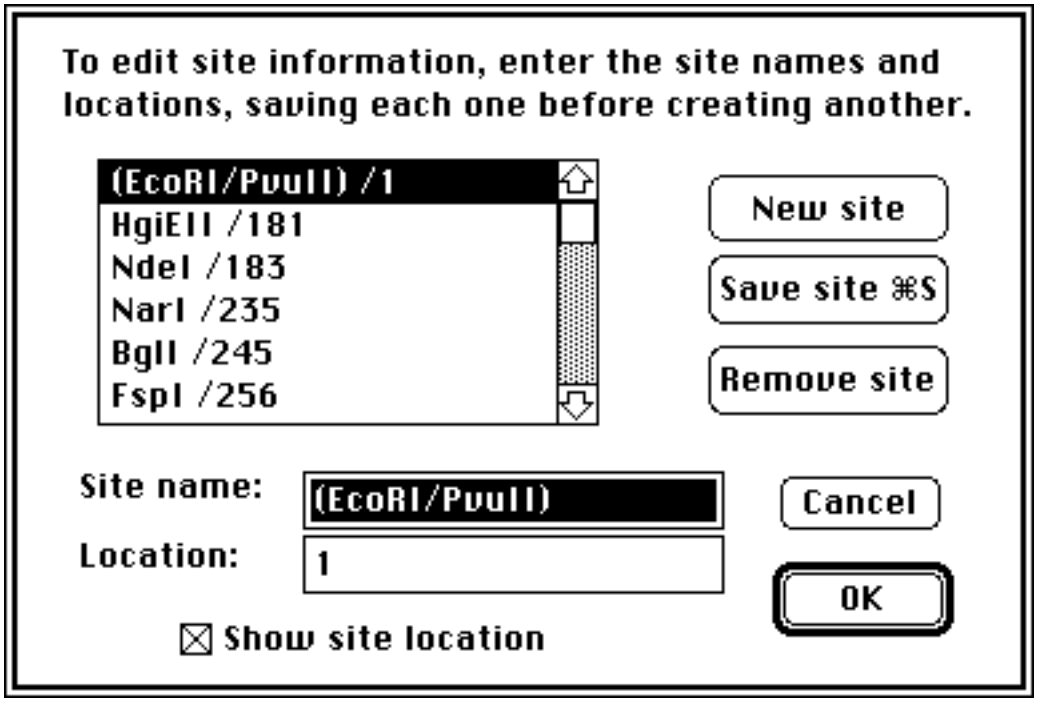
Site editing

Restriction enzyme recognition sites (or any other sites of particular importance) may be edited with the site editing dialog box shown below. To activate this dialog box, one of the following three methods may be used (also described in the last section, OVERVIEW OF MacPlasmap):

1) Click outside the plasmid circle, drag to the desired location (indicated by a flashing number), then release the mouse and click in the flashing box. Select the **Add Site...** item when a popup menu appears.

2) Click in the site editing icon on the left side of the window.

3) Choose **Sites...** from **Data** menu.



All site editing is done within the site dialog box. Enter site name (default **New**) and location (automatically displayed if the flashing box was on prior to activating the dialog box), then click the **Save site** button (or use Command-S), or the **OK** button. A bold line appears below the **Save site** button when the location number is valid, otherwise the number will not be recorded.

Clicking the **New site** button restores the default site name and location, in preparation for entry of new sites.

To remove a site from the map, you need to first select the site from the site list (by clicking on it), and then click the **Remove site** button.

Warning Removed sites cannot be recovered by clicking the **Cancel** button.

Selecting a site from the site list also displays its current attributes in appropriate fields (name, location and show/hide site position). You can make changes and save them by clicking the **Save site** button. The old site will be replaced by the changed one. If you prefer to draw the position along with the site, you should check-on the **Show site position** box.

When editing is finished, clicking the **OK** button will cause re-drawing of the plasmid map on screen, with the new data incorporated.

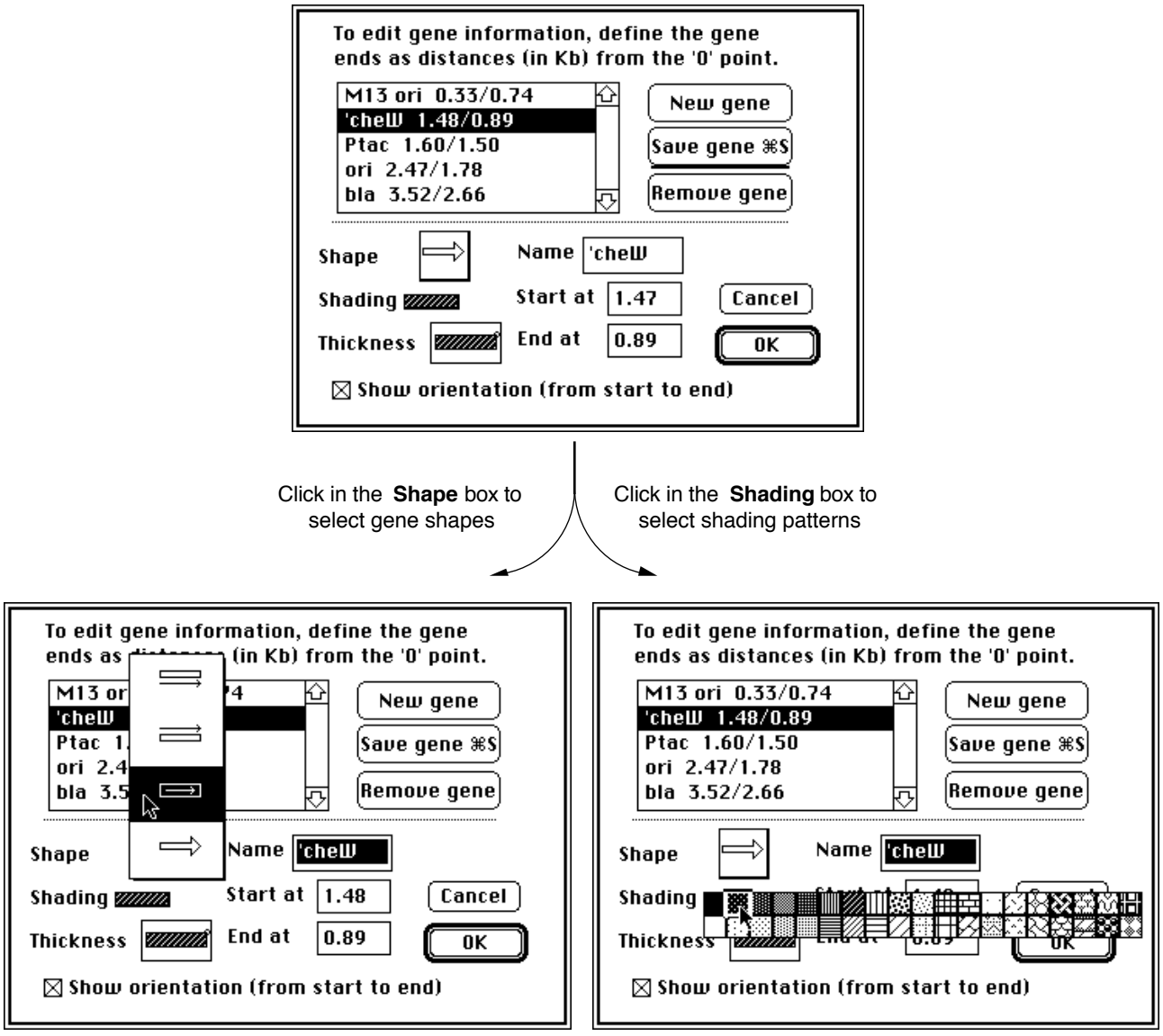
Note that removal of sites (or genes) from the plasmid does not change the size of the plasmid. It only removes them from the site (or gene) list. To actually delete a DNA fragment from the plasmid, use the deletion editing described later in this section (page 15, **Deletion and Insertion**).

Sometimes sites can overlap when many are clustered in one region of the circle. You can remedy such problems either by using polylinkers or by shifting their positions with the adjustment tool (the hand-shaped cursor).

> *Note* 1) The maximum number of sites in a plasmid map is limited to 60.
> 2) You can press the TAB key to quickly move the cursor from one text editing field to another. When combined with the Command-**S** keys for save, this general technique allows fast data input within dialog boxes.

Gene editing

Genes (or other contiguous segments of interest) can be edited in a way similar to site editing. The three buttons, **New gene**, **Save gene**, and **Remove gene**, work exactly the same way as the corresponding buttons in the site editing dialog box. Other attributes specific to genes are described below and illustrated in the following diagram:



Any of the following attributes can be specified to either a newly added gene or a pre-existing one after it is selected from the gene list.

Shape. Four different types of arc are available for drawing genes. They are listed under a pop-up menu which can be activated by clicking on the box displaying the current shape.

Shading. Shading patterns are chosen from 38 standard patterns on a popup menu, which can be activated by clicking the small shading box.

Thickness. The thickness of a gene segment can be changed by clicking and dragging the small circle located at the top-right corner of the thickness rectangle.

Orientation. If the **Show orientation (from start to end)** item is checked, an appropriate arrow will be drawn with the gene to indicate the orientation of the current gene. The type of arrow is determined by the gene **shape** attribute. The arrow is always drawn from the start to the end point of the gene.

Note There are two limitations of gene editing in the current version of MacPlasmap: 1) Genes are not allowed to go across the 'O' point. 2) The maximum number of genes in a plasmid map is 30.

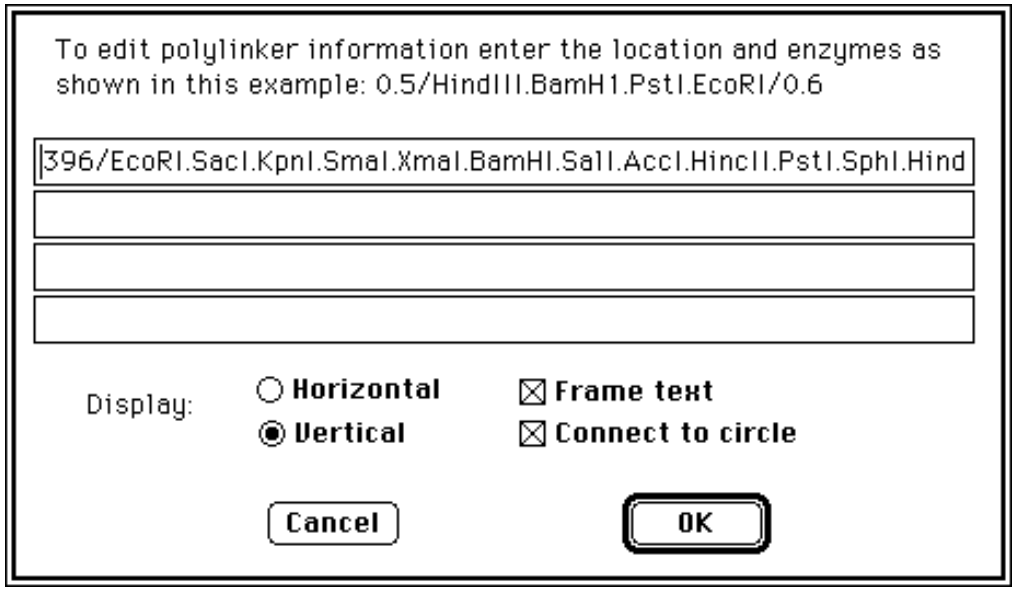
Polylinker editing

Polylinkers should be used to group restriction sites that are too crowded to be drawn separately. Use the same techniques as for editing sites and genes to activate the polylinker editing dialog box, and then enter the data as given in the example shown in the dialog box. The polylinker format, which requires that the start position and the sites of a polylinker be separated by a slash (/), must be matched for successful data entry. An optional endpoint can be applied to specify a segment on the plasmid circle upon which the polylinker is attached (provided that the **Connect to circle** box is checked on). Without this value, the polylinker is drawn as a single site on the circle.

By convention, sites in a polylinker are ordered clockwise, and the polylinker location is the position of the first site in the linker.

You can control the appearance of a polylinker with four parameters in the dialog box. Polylinker on the screen can be moved by using the adjustment tool as for any other text items.

An example of polylinker data is shown below:



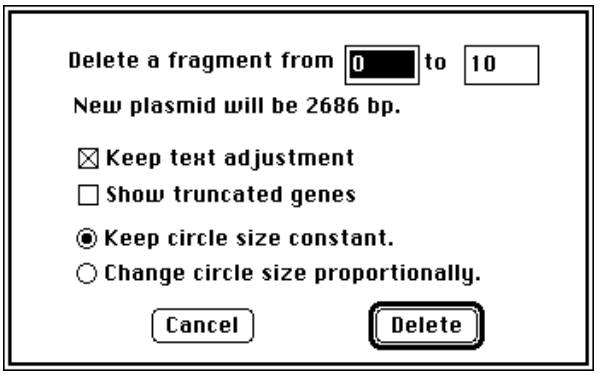
Note A maximum of four polylinkers is allowed for each map.

Deletion and insertion of fragments

MacPlasmap allows the deletion and insertion of fragments on plasmid maps. These are often necessary for drawing new maps from pre-existing ones. These two functions are performed through the use of other two dialog boxes, which can be invoked by any of the three methods already described for other editing operations. Note that the option **Show truncated genes** is currently not supported. Therefore insertions into a gene, or deletions ending or starting within a gene will remove that gene from the gene list. Sites and polylinkers affected by this operation will also be removed. By definition, a site is affected if it is included in the deleted fragment, and a gene is affected (inactivated) when one of the following is true.

- The site of insertion is inside the gene.
- At least one of the deletion endpoints is inside the gene.
- The deletion completely covers the gene.

Note Deletion or insertion of fragments changes the size (in kb or bp) of the plasmid. This will cause necessary re-drawing and re-adjustment of text items associated with the map circle. If the changes are small and you prefer to keep the original text positions, you can check the **Keep text adjustment** box (see below). This check box is turned on by default to prevent accidental loss of adjustment. If the changes are significant and the original text adjustments no longer apply, you can choose the **Calculate text positions** command from the **Edit** menu to re-calculate test positions and then re-adjust them by using the adjustment tool.



These procedures are also recommended when you change the plasmid size from the **Overall parameter editing** dialog box (page 16, next section).

Map parameter editing

MacPlasmap uses a number of map parameters to control the overall appearance of a plasmid map. Through the use of the map parameter dialog box (which is essentially the same as the **New...** dialog box, page 7), the following aspects of a plasmid map can be changed:

1) Plasmid name. It is often necessary to change the name of a plasmid when it is derived from modifying a previous map. Changing the plasmid name will also have the following two effects: a)The title of the window containing the map will be changed to the plasmid name, b)The corresponding item under the **Windows** menu will show the new name.

Note Since changing the name of the current plasmid signifies the creation of a new plasmid, this change is recommended as the first step of producing a new map from an old one.

2) Plasmid size. This should not be changed unless absolutely necessary. Modifications like insertion and deletion of fragments automatically updates this attribute.

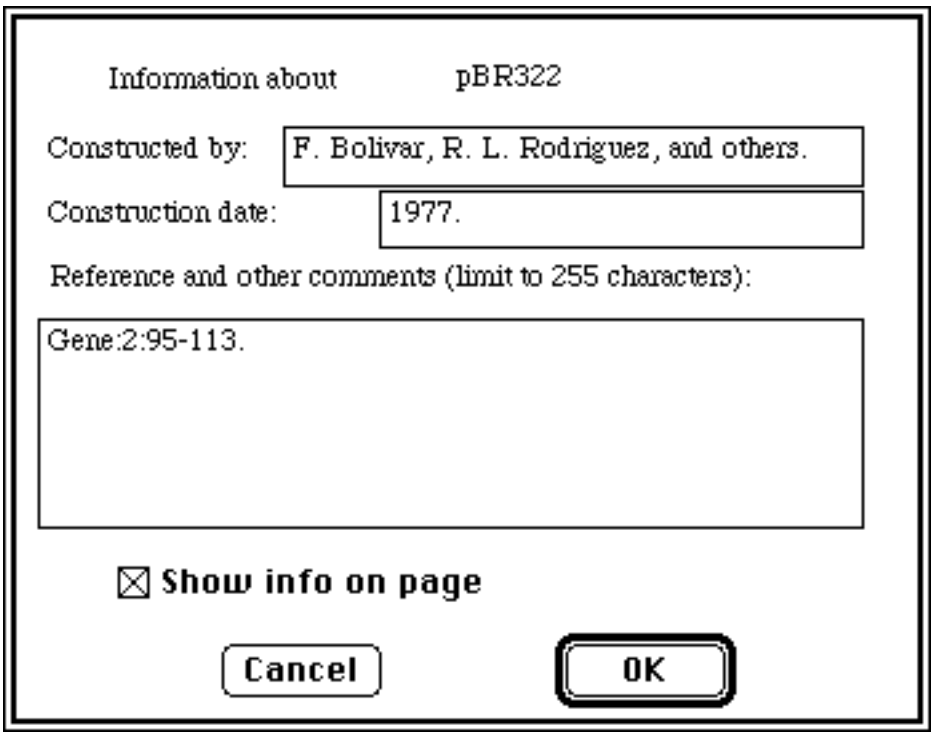
3) Map circle diameter and thickness. Change these to control the size and the thickness of the map circle.

4) Plasmid type. Switch between two types of maps.

5) Site positions. The **Show site position as default** check box works the same as in the **New...** dialog box described in page 8. This item does not directly affect the drawing of sites, but merely changes the default value of the **Show site position** check box in the Site editing dialog box.

Reference/Comment Editing

Information about the plasmid can be entered with this dialog box (shown below). Note that the maximum length of a reference is 255 characters. References longer than that will be truncated. The **Show info on page** box is checked by default which allows the plasmid information to be included as part of the map. Un-check this box if you don't want this to occur. The text can also be repositioned by using the adjustment tool.



Saving and retrieving map data

You can save the maps you created in two different types of files: MacPlasmap data file and picture (PICT) file. Use the **Save** or **Save As...** command from the **File** menu to save a data file. Only data files can be retrieved by MacPlasmap. Maps saved as PICT files by using the **Save As PICT File...** command will not be recognized by MacPlasmap, but can be opened by most commercial Macintosh drawing programs, thereby providing a way of communicating with other programs. The same kind of transfer can be done by copying the map from MacPlasmap (using **Copy Map to Clipboard** from the **Edit** menu), and pasting it into other programs.

> *Caution* MacPlasmap is capable of drawing very fine hairlines on LaserWriters, a function that some drawing programs do not seem to have. Printing plasmid maps with other drawing programs may produce thicker lines than if the maps are printed directly by MacPlasmap.

Plasmid data files can be retrieved by simply choosing the **Open...** command from the **File** menu. An opened plasmid map will be displayed in a new window. As many as 15 maps can be displayed concurrently within the program.

Printing Plasmid Maps

Plasmid maps can be printed on either Apple LaserWriters or ImageWriters, by choosing the **Print...** command from the **File** menu.

> *Caution* When using ImageWriters, be sure to select **Page Set Up...** first, and choose '**Tall Adjusted**' as Special effect. If this is not done, plasmid maps will not print as true circles.

MacPlasmap REFERENCE

This section briefly summaries the MacPlasmap menu commands, many of them have been detailed or mentioned in previous sections.

Apple Menu

About MacPlasmap... Shows the version number and memory available for the program.

File Menu

Help/H... Shows an on-line help screen explaining some basic operations of MacPlasmap.

New/N... Opens a new window for a new plasmid.

Open/O... Opens plasmid data files from disk. Data files must be created by MacPlasmap using the **Save** or **Save As...** command.

Close/W**.** Closes the front-most window on the screen. If the current plasmid data have not been saved, a warning dialog is given before closing is allowed.

Save/S. Saves data file using the same name as when the file was last saved. The old file with the same name is overwritten.

Save As... Saves the data file with a new name. The standard file dialog box is presented.

Save As PICT File.../G. Saves data to a PICT file with a new name. PICT files can **not** be read and modified by MacPlasmap, but can be opened by most commercial drawing programs.

Page Setup... Sets up printing pages. It is important to do this every time when changing printers.

Caution Be sure to check the '**Tall Adjusted**' option in the **Special Effects** section of the dialog box when using an ImageWriter or ImageWriterII. This will ensure that maps print as true circles.

Print/P. Prints plasmid maps on either ImageWriters or LaserWriters.

Transfer/T. Transfers to any other application directly from MacPlasmap without returning to the desktop.

Quit/Q. Quits MacPlasmap.

Edit Menu.

Undo/Z. Not supported in MacPlasmap. For use by desk accessories.

Cut/X. For use by desk accessories.

Copy/C. For use by desk accessories.

Paste/V. For use by desk accessories.

Clear. For use by desk accessories.

Copy Map to Clipboard/M. Copies the current plasmid map to the clipboard as a picture which can then be pasted into other drawing programs or into the scrapbook.

Text font. Displays a list of fonts available on your system. You can choose one of them as the text font in a plasmid map. Currently used font in the map is check-marked.

Text size. Displays a list of font sizes you can use to specify the text size in a plasmid map. As with **Text font**, currently used size is indicated by a check-mark.

Calculate text positions/A. Re-calculates the positions of site names on the screen. All previous adjustments will be discarded. This command is commonly used when the plasmid size is changed (by deletion or insertion of a fragment, for example) .

Data Menu.

This menu contains seven items corresponding to the seven editing functions of MacPlasmap. Selection of any item will bring up a dialog box for data entry. Choosing items from this menu is only one of the three ways to enter map data. It gives you the option of using keyboard commands (æ-1 to æ-7) instead of clicking and dragging with the mouse.

Windows Menu.

The **Windows** menu provides a means for organizing multiple windows on the screen. All window titles, which are the same as document names, are listed under the **Windows** menu. Choosing one item from the list brings the corresponding window to the front. This is an easy way to activate a particular window when many windows are open. The item corresponding to the frontmost window is always check-marked.

COMMENTS AND SUGGESTIONS

Your comments are vital to the improvement of MacPlasmap. Please send your suggestions and/or bug reports to

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Your comments may include (but not limited to): overall usefulness of MacPlasmap, program bugs and their descriptions, features you like and dislike, functions you want to be added, etc.

Program version:

Comments:

Name:_____ Address:_____

City:_____ State:_____

Contry:_____ Phone #:_____