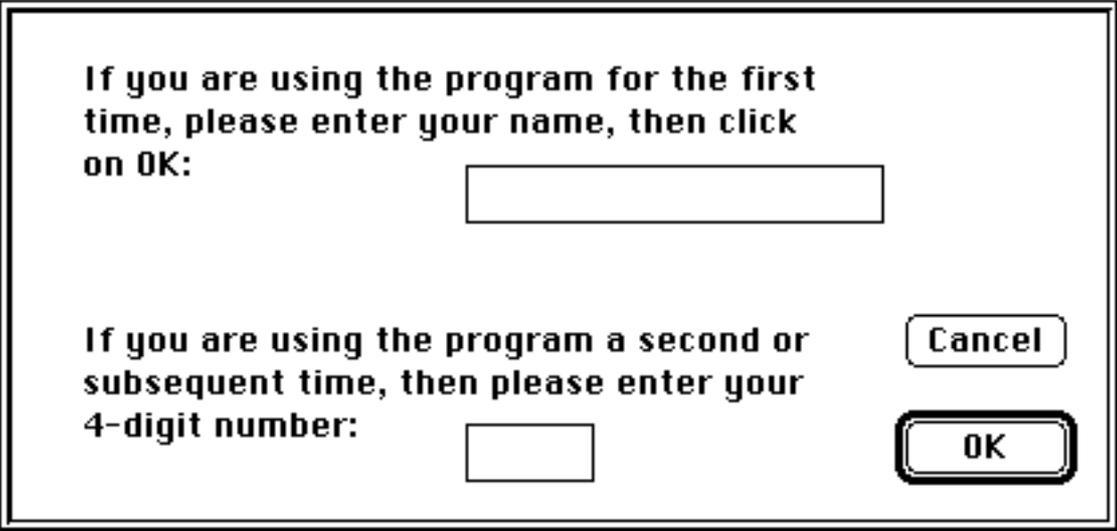


The Gelsim Program

Gelsim is a program which simulates the process of producing a restriction map of a DNA molecule. Using this program you will cut the DNA, run gels, measure fragments, and piece together a map showing where the restriction enzymes cut.

Starting the exercise

You activate the program by double-clicking on the Gelsim icon, or alternatively by clicking once on the icon and choosing Open from the File menu. After the introductory screen, the computer will show a dialog box asking you to input your name, which will look something like the following:



If you are using the program for the first time, please enter your name, then click on OK:

If you are using the program a second or subsequent time, then please enter your 4-digit number:

Cancel

OK

For the moment you should just type your name, which may be in any format, and click the mouse button while the pointer is on the **OK** button. This button is highlighted, which means that pressing the **Return** or **Enter** keys will have the same effect as clicking on the button.

The program begins by converting the name into a number. This number will be shown in a second dialog box, and it is important that you note down this number during this time. It is this number which is used to generate the sequence you will be working on. If you need to regenerate the sequence at a later stage, you should use this number to re-start the exercise, rather than the name. Also if you hand in a report for the exercise, the number will be required to check the restriction map which you obtain.

The menus

The selection of menus in the program is as follows:

File **Edit** **Workbench** **Photos** **Help**

Most of the commands needed for this program are in the **Workbench** menu. Commands in this menu are grouped in four headings as shown below:

(1)	(2)	(3)	(4)
Pour a gel	Cut DNA	Run the gel	Throw out gel
	Size markers	Activate gel cutter	
	Pick gel slot	Cut out fragment	
	Load DNA		
	Throw away DNA		

All operations with photographs (see below) are handled by the **Photos** menu. Other commands which are available at all times are contained within the **File** and **Help** menus. The **Edit** menu is inactivated and is only included for compatibility with Macintosh standards.

Getting started on the project

Initially, when you have started the program and entered your name, you will be confronted by a blank screen, with no clues about what you are to do. The only thing which you can do is to scan the different menus, particularly the **Workbench** menu which is the main working menu. Doing this, you will find that the only command which is highlighted on the **Workbench** menu is the first command, **Pour a gel**. Nothing else works until you do this.

This scanning of the menus illustrates an important point about the way that the program works. At each stage, there will be one or more actions which are appropriate. These appropriate options are activated, ie. not dimmed, in the menus. The first thing which needs to be done is the pouring of a gel. Once you have done this, you will find that other commands become enabled and therefore available.

Some menu options are available at all, or nearly all, times. For example, the options of quitting (**File** menu) or throwing away the gel (**Workbench** menu) are available at most times. The items on the **Help** menu are similarly available at all times.

Digesting DNA

In real life, the cutting of DNA by restriction enzymes takes a considerable amount of time. You would normally run several digests at the same time. While these digestions are running, you might pour a gel. It would certainly not be efficient to wait until one reaction has finished before beginning the next.

This computer program works in a slightly different way. For simplicity, it is assumed that cutting of a DNA sequence and loading it onto a gel are operations which occur sequentially. Once one sequence has been cut and loaded, you then start on the next.

Having poured a gel, you will now see that the **Cut DNA** and the **Size markers** commands are activated. If you activate the **Cut DNA** item, you will see the dialog box shown below.

You don't have to do anything except click on the restriction enzyme with which you wish to digest the DNA. You are limited to cutting with either one or two enzymes, or you may choose not to cut at all.

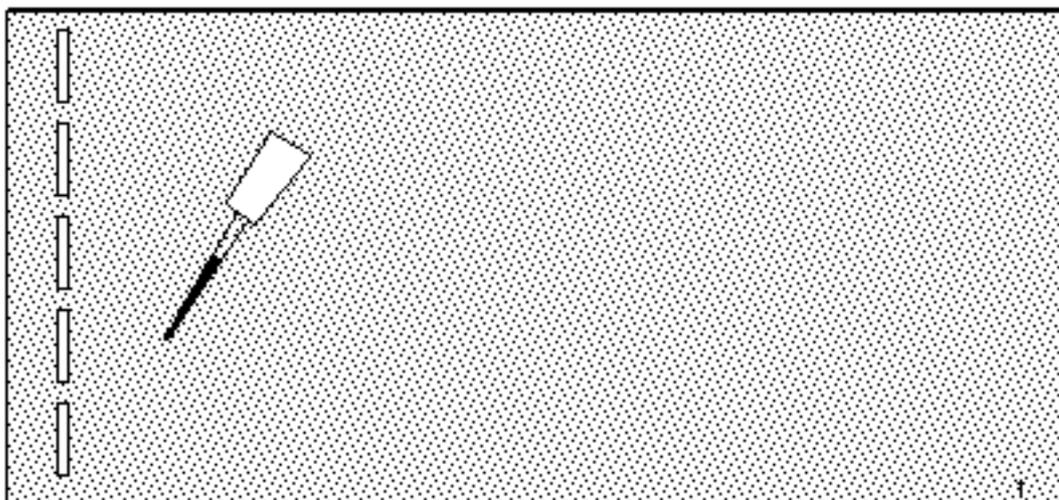
Nominate enzyme(s) to cut your DNA

Apal
 BamHI
 ClaI
 DraI
 EcoRI
 HindIII
 KpnI
 MluI
 NcoI
 PstI
 Sall
 HhoI

Complete digest
 Partial digest

If you click on one of the enzymes, the **Complete / Partial** digest choice will be activated. Partial digests in this case are guaranteed to produce not just the fragments between adjacent markers, but all combinations of fragments between each cutting site.

Once DNA has been cut or size markers selected, a pipette containing the cut DNA will appear, and the **Pick gel slot** command is activated:



You need to move the mouse button over the pipette, and drag the pipette so that the tip is over a vacant slot. The **Pick gel slot** command does not actually do anything except tell you what to do. You can use the cursor keys (up, down, left, right) for fine control.

If the tip is over a vacant slot, the **Load DNA** command will be activated. Use this command to put the DNA into the gel slot and dispose of the pipette. The Enter or Return keys will also have the same effect at this stage. Alternatively, if you have changed your mind and decided not to use that sample, move the pipette off the gel and activate the **Throw away DNA** command (not recommended in real life).

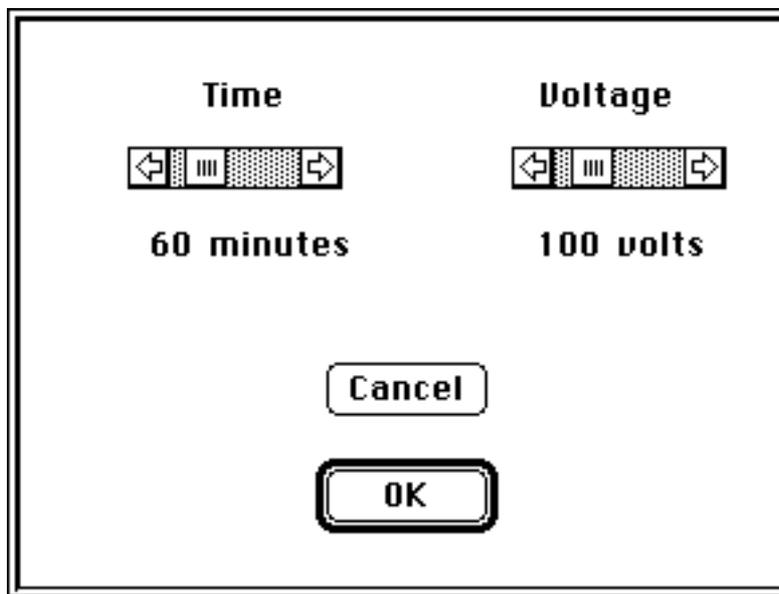
Marker DNA

As an alternative to cutting the unknown DNA, you may run samples of marker DNA of known sizes. Three markers are available as follows:

SPP1/EcoRI	λ /EcoRI
8510	21800
7350	7520
6110	5930
4840	5540
3540	4800
2810	3410
1950	
	λ /HindIII
1800	23700
1510	9416
1390	6557
1160	4361
980	2322
720	2027
480	564
360	125

Running the gel

Once DNA samples have been loaded into one or more lanes, running the gel becomes an option. Accessing this option on the menu will bring up the following dialog box. If you click the OK button, the gel will run at the standard times of 60 minutes at 100 volts. You can, if you wish, change either of these values. As standard scroll bars, you can change values in any of three ways: (a) clicking in the arrows, which gives the finest control, (b) clicking in the scroll bar to the left or right of the movable box, (c) dragging the box. Clicking in the **OK** button then confirms any changes you have made. Clicking the **Cancel** button, as in most cases, dismisses the dialog box without any action taken.



Get info

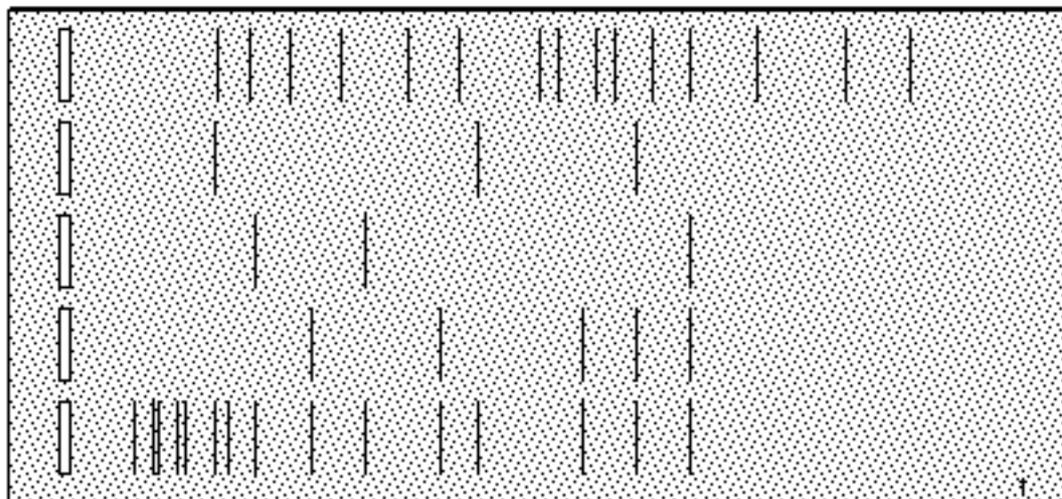
You will find a description of the current gel from the **Get info** command. The diagram below shows one example of such a window:

<input type="radio"/> No gel			<input type="radio"/> Gel run, not photographed
<input checked="" type="radio"/> Gel loading			<input type="radio"/> Gel run and photographed
Track	DNA	Enzyme(s)	
(1)	SPPI	EcoRI	Name John Smith
(2)	Whole DNA		Number 8803
(3)	Whole DNA	BamHI	Gel No. 4
(4)	Whole DNA	EcoRI	
(5)	Whole DNA	BamHI EcoRI	
(6)	Whole DNA	BamHI EcoRI (P)	
(7)			
<input type="button" value="Show size markers"/>			<input type="button" value="Cancel"/>

The top radio buttons show the current status of the gel. In this case the gel is loading and not yet run, with seven tracks, the last one currently empty. The first track has size markers. The remaining tracks are loaded with the unknown DNA, uncut in the second track, digested with either one or two enzymes, including a partial digest in track 6. The right-most column shows the user name and number, the number of the gel currently being run, and the **Cancel** button to dismiss the window. The **Show size markers** button brings up a window with the list of size markers shown previously.

Taking photographs

If you run a gel, you may see something like the following:



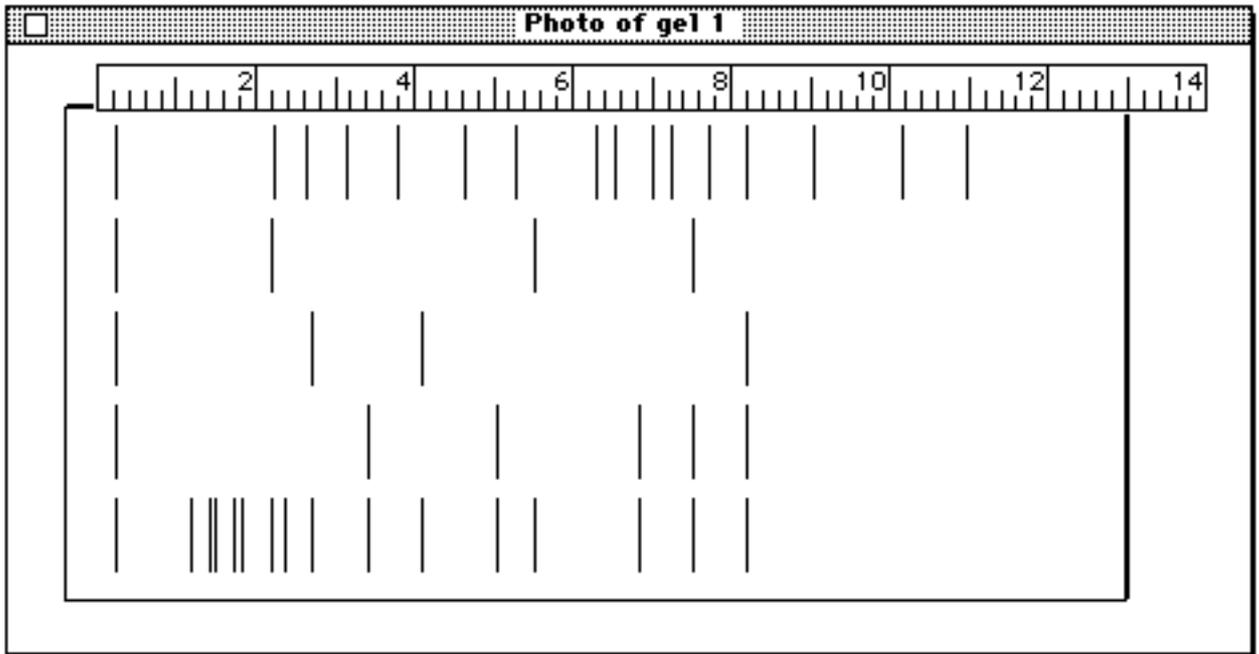
There are various bands, whose length you will probably need to measure. This is best done by taking a photograph, which preserves the results for later reference and allows the bands to be better visualised. Choose the **Take a photo** item on the **Photos** menu. An alert box will tell you that a photograph has been taken. Click anywhere to make this box go away. From this point onwards, you may look at the photograph at any stage using the **Retrieve photo** item. In the present case, the photo would look as shown on the following page.

Bands can be measured using the ruler, which can be moved anywhere over the photo. Use the cursor keys for fine control.

While a photo is shown on the screen, only the **Put away photo** item is active. Nothing else can be done until the photo is put away. The last item on the **Photos** menu, **Throw out photo**, will only become active if the maximum number of stored photographs (20) is exceeded.

Cutting out fragments

The small object above and to the left of the gel is the gel cutter. Move it over any fragment you wish to cut from the gel, and access the **Cut out fragment** command. A dialog box will appear showing the number associated with that fragment. For example, the gel above is Gel 1 (numbered in the bottom right corner), and the first fragment cut from this gel would be assigned the label 1.1. You need to exercise care in noting down where these fragments come from.



If you have cut out fragments from a gel, the program recognises this when you come to run the next gel (after you have thrown out the old gel) by changing the form of the dialog box to allow you to choose from the available fragments. In the above case, you would get a dialog box as shown below:

(1) Nominate DNA (complete sequence or fragment)

(2) Nominate enzyme(s) to cut your DNA

(3) Complete or partial digest

(1)

Whole DNA
1.1

(2)

- Apal
- BamHI
- ClaI
- DraI
- EcoRI
- HindIII
- KpnI
- MluI
- NcoI
- PstI
- Sall
- XhoI

(3)

Complete digest

Partial digest

Cancel

OK

As well as choosing the restriction enzyme(s) and Complete / Partial, you can also nominate the DNA in this case. The original unknown DNA is chosen by default. There is only enough DNA in each sample for two tracks. Once you have used up the fragment, the following boxes will show this item as dimmed.

Samples may be cumulated from one gel to the next. However there is only room to store 20 samples, so that if you reach this limit you will have to start using some before others can be added.

Tutorial

A tutorial may be available, giving examples of some aspects of restriction enzyme mapping. Access this from the second group of the **Help** menu. As with the **Help** screens, you can go back and forwards through the tutorial, and must quit the tutorial before doing anything else. Unlike the **Help** screens, you can only start the tutorial at the beginning.

Trial run

The program gives you considerable flexibility in running trial examples. You can run these at any stage, but must interrupt any work done on the unknown sequence, since the program can only hold one sequence at a time.

When you access **Trial** from the **Help** menu, you will see a similar blank screen to that shown when the program first starts, except that there is an extra menu called **Answers**. The last item in this menu, **Show map**, is what you use to show the true map. The trial item has also changed to **End trial**, and there is a new item called **New sequence** at the bottom of the help menu.

Naturally you will get more out of the trial as a practice if you try to work out the map before looking at the answers. However there is nothing to stop you looking at answers at any time while in trial mode, and you can always call on another sequence if you have looked at one.

When you access **Show map**, a menu will appear which allows you to nominate which enzymes are to be shown in the map. A single check box also gives you the opportunity of nominating all enzyme sites. The map which is produced may, however, be a little difficult to interpret if there are many enzyme sites close to each other.

The menu also gives you the choice of nominating one of the enzymes as the apex (12 o'clock) of the map. This only takes effect if the map is circular. If you have already drawn a map using one such enzyme as the apex then it makes sense to choose this to check on your map.

Once the map is showing on the screen, many other options become available on the answers menu. The map can be reversed, either for a circular or linear map, using the **Flip** item. If you have a circular sequence, the map can also be rotated to use other occurrences of the nominated enzyme at the apex.

The map can be printed out. Just use the **Print** command from the answers menu. It can be saved to a file in two different ways. The first way is as a picture (PICT) file, which can be accessed from many drawing or word-processing programs. The second way is as a text file (TEXT), in which the map positions and distances are listed out sequentially. This type of file can be read and/or altered from most word-processing programs.

While the map is shown on the screen, most other items in the program are inactivated. You will see that the bottom item in the **Answers** menu has now become Hide map, and you must do this before going back to do anything else.

In order to get back to try the unknown sequence, you will eventually have to access the **End trial** menu item, which is itself inactivated while a map is shown on the screen. Alternatively you can quit from the **File** menu, and re-start the program.

Some rules and restrictions of the program

Nothing can be done unless a gel has been poured. The number of tracks on a gel may be from 4 to 7. Only one gel may be run at a time, so that the old gel must be thrown away before a new one can be poured.

The migration distance of fragments on the gel may be controlled either by changing the time of run or the voltage. Replicate runs of the same time and voltage run at exactly the same rates, so that the gel will only need to be calibrated once. The distance migrated on the gel follows a log-linear relationship with size, except for the very large lambda molecules. Circular molecules may run either a little slower or faster than the equivalent sized linear molecule.

Photographs may only be taken immediately after a gel has been run. Photographs may be retrieved for measurement at any stage, although only one photograph may be shown on the screen at a time. A total of 20 photographs may be stored at any one time. When this limit is reached, a dialog box will ask you to remove old photos before new ones can be added.