Internet On-Ramp

A Virtual Molecular Biology Teaching Laboratory

Robert M. Horton, Attotron Biosensor Corporation, Carson City, NV, USA (rmhorton@attotron.com) and Robert C. Tait, Biotechnology Consultant, Davis, CA, USA

Recombinant DNA technologies and genetic analysis have become such an important aspect of technological culture that the analysis of disease-related genes, production of mammalian hormones and antibodies in bacteria, the use of DNA evidence in criminal proceedings and mammalian cloning all appear routinely as news items. The economic and social impact of molecular genetics has created a demand for training in these areas that extends well beyond the confines of research laboratories (1). Laboratory researchers can expect to be called upon to teach these technologies with increasing frequency to students from an ever broadening spectrum of disciplines. However, the expense and technical demands of maintaining biological strains, enzymes and DNA samples for even simple laboratory exercises can be burdensome and can severely strain educational budgets.

Here we present the restriction analysis module of the Cybertory™ Molecular Biology Laboratory Simulator, which is being developed in an effort to reduce the costs and technical demands associated with presenting training in principles of molecular biotechnology to a broad audience. This program is written in client-side JavaScript™, and runs in the Netscape Navigator web browser (Version 4.03 or higher). It can be run either from the Internet or on stand-alone machines (Macintosh® and Windows®).

Launching the Laboratory

Start the program from the start page (<http://www.attotron.com/cybertory/start.htm>). Buttons let you choose a work area size. The program is designed to use a 600 × 800 pixel area; however, it can be launched in 640 × 480 mode for display on a low-resolution monitor or television set or in a larger window to cover the desktop.

Experimental parameters such as the available restriction enzymes and DNA sequences are set by placing an "order" with BOBCO, a fictitious Virtual Supply Company.

BOBCO has an on-line (or off-line, for that matter) catalog, where one can find technical information about the various "products", such as restriction enzyme recognition sequences, the sizes of the bands in the molecular weight marker, etc. Placing an order sets client-side cookies that remember your choices from one computer session to the next. An order specifies four restriction enzymes and six DNA samples.

The BOBCO order form is password-protected, so that you can choose whether or not to let your students change the experimental parameters; give out the password if you want them to order their own enzymes and/or DNA samples. The secret password is Laguna.

Figure 1 illustrates the interface for setting up the reactions. The example shown here uses the "Sickle Cell Anemia Genetic Diagnosis Sample Kit", which includes linear DNA samples (virtual PCR products) from one positive control individual homozygous for the sickle cell mutation (SS), one negative control homozygous wild type (WW) and four individuals of unknown genotype (Pt1–Pt4). The goal of the experiment is to determine these unknown genotypes.

Twelve reagent tubes are arrayed on the left side of the screen, and twelve reaction tubes are arrayed on the right. Clicking on a reagent tube pops its top open, and the micropipettor travels to the selected tube. Click on the volume control wheel to increase volume (right side to increase, left to decrease), or select the volume display with the mouse and type a new value. Draw fluid into the pipet by clicking on the plunger. To move the material to another tube, click on the target tube; its top

Figure 1. Restriction enzyme reaction setup. Clicking on tubes moves the pipettor, allowing mixing of ingredients from the reagent tubes on the left into the reaction tubes on the right.

Internet On-Ramp

will pop open, and the pipettor will move to the newly selected tube. Click on the plunger again to dispense the pipet contents.

Click on the tip ejector or the waste bin to eject a used tip. Click on the tip ejector again (or the tip box) to get a new one.

When all reaction ingredients have been added, the **Start** button of the timer begins the incubation. The contents of the tubes will be fully digested with whatever enzymes were added (no partial digests are allowed).

Gel Analysis

To determine the sizes of the restriction fragments, choose *Agarose Gel* from the pull-down menu at the upper right corner of the screen (Figure 2). The **Auto Load Gel** button on the transilluminating gel box causes the twelve reaction tubes to be loaded into the gel wells from left to right, with molecular weight marker in the thirteenth well. Set an appropriate voltage and time on the power supply (BOBCO gels never melt), then click on the **on/off** button to begin electrophoresis. A red light on the power supply and bubbles from the electrodes confirm that the power is on, and the blue loading dyes migrate through the gel. To see the DNA,

BioBit: Other "Virtual Laboratory" Sites

BioMOO Environmental Genetics Laboratory - Ames assay (<http://bioinfo.weizmann.ac.il:8888>). See http://www.liv.ac.uk/ctibiol/vCUBE97/html/zev_leifer.html for description and instructions.

Howard Hughes Medical Institute - ELISA Assay (<http://www.hhmi.org/grants/lectures/vlab1/>), Interactive Shockwave animations.

Microbiology 51 - Interactive Pathogenic Microbiology (<http://www.koonts.com/micro51/cases.html>), HyperText-based guess-the-next-step tutorial.

turn the UV light on and the room lights off.

Measure migration distances by clicking on and dragging the fluorescent ruler. Turn the room lights on briefly to line up the ruler with the bottom of the gel. Plotting migration curves has been left as a student exercise.

Though the catalog has several pre-designed "kits", the experiments themselves are not built into the simulator. The *custom DNA synthesis* option lets you combine any sequence (within your computer's memory limits) with your choice of restriction enzymes to create your

own experiments. This may be useful for "dry-labs" with which students can practice before undertaking the more laborious, more expensive, and less reliable real thing.

Molecular biology in the real world demands sophisticated equipment and supplies, technical expertise and manual dexterity. The goal of the virtual molecular biology laboratory is to lower these barriers and facilitate teaching of key molecular genetics concepts.

ACKNOWLEDGMENT

Development of these programs was supported by Grant No. 1 R43 RR13645-01 from the National Center for Research Resources.

REFERENCES

1. Tait, R.C. 1997. An Introduction to Molecular Biology. Horizon Scientific Press, Wymondham UK.
2. Husain SM, P. Kalavathi, and M.P.J.S. Anandaraj. 1995. Analysis of sickle cell gene using polymerase chain reaction and restriction enzyme Bsu 361. Indian J. Med. Res. 101:273-276.

Figure 2. Gel electrophoresis. Six virtual DNA samples representing 771-bp PCR products of human genomic β -globin have been cut with the restriction enzyme *Bsu*36 I. Samples are run in two concentrations. Lanes 1 and 7: homozygous sickle-cell; lanes 2 and 8: homozygous normal; lanes 3-6 and 9-12: patients being tested. The normal gene yields bands of 254, 228, 201 and 88 bp. The mutation destroys a *Bsu*36 I site, giving bands of 429, 254 and 88 bp. Lane 13 contains a 150-bp ladder, with bands from 150 to 3000 bp. Ladder bands are equimolar, so larger bands contain proportionately more DNA. Starting sequences were taken from GenBank[®] locus HUMHBB using the primer sequences from Reference 2, but are 1 bp shorter than described in Reference 2.