PCR Master Mix Volume Calculators in JavaScript™

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When setting up multiple polymerase chain reactions (PCRs), it is often a good idea to make a “master mix” containing all of the common ingredients, which can then be added to each reaction tube in a single pipetting step. Individual ingredients (such as templates for different reactions) can then be added to each tube. Using master mixes saves pipetting steps (thus saves time and money) and increases the accuracy of reactions by avoiding the measurement of extremely small (perhaps sub-microliter) volumes of reagents that need to be added to a single tube.

Calculating the amount of each ingredient to add to a master mix can be a chore, especially if you are doing two-dimensional titrations where varying concentrations of two ingredients are used in different combinations and you want to make one big master mix to contain the ingredients for the sub-masters, which will themselves be further divided. Each nested step must include a “fudge” factor to allow for pipetting errors; this ensures that you don’t run short on the last tube. Good calculations using a reasonable fudge factor save reagents more so than using a fudge factor that is easy to calculate. In other words, a quick and dirty approach might be just to add enough extra ingredients for an additional reaction. However, a fudge factor based on a percentage of the total volume is probably more appropriate; one extra reaction might be excessive if you are only making four reactions, and it might not be enough if you are making 96.

Computerized Worksheets

Setting up a worksheet to calculate master mix ingredients is very helpful to keep everything straight. Generic worksheets including the standard amounts of each reagent for one reaction together with a column of blank lines to enter the calculated amounts needed for a set of reactions (including a fudge factor) are very useful and also give you a record to put in your notebook. Computers are, of course, ideal tools for performing such calculations, and a worksheet on a computer gives you the best of both worlds. A program for performing such calculations using a macro for Microsoft® Excel® has been published (1). This approach has several interesting points from a programming and user’s perspective. A macro is a set of commands that are executed inside of another program; in this case, the macro program runs inside Excel. The remarkable thing about this approach is that it doesn’t matter what kind of computer the user has as long as it runs Excel (which is available for both Windows® and Macintosh®).

Unfortunately, a macro for a program like Excel suffers from some drawbacks that plague many pre-World Wide Web (WWW) computing approaches. Users must have Excel (which can be rather expensive, even with an academic discount), obtain the macro (download it or get it on a floppy disk), install it and figure out how to use it. Ironically, such obstacles can cause people to use their multi-hundred-megahertz computers to print out a worksheet, which they fill in

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Out of primary antibody? If your Western procedure includes a lag between the time of protein transfer to membranes and incubation of membranes in primary antibody, netters recommend washing and air-drying the membrane, followed by short-term storage in a dry place at room temperature; long-term storage should be at -20°C.

Netters providing tips on improving the performance of long PCR recommend that phosphorothioate linkages be included in the final few nucleotides at the 3’ ends of primers to prevent primer “editing” by proofreading polymerases such as Pfu. This concept is discussed in A. Skerra, 1992, Phosphorothioate primers improve the amplification of DNA sequences by DNA polymerases with proofreading activity, Nucleic Acids Res. 20:3551-3554.

Seeking information on the properties of a particular enzyme, a netter was referred to the enzyme manual on the Web site of Worthington Biochemical Corporation at www.worthington-biochem.com

BioCitation

This procedure was recommended by netters as a reliable method for stripping and reprobing Western blots: J. Tesfaigzi, W. Smith-Harrison and D.M. Carlson, 1994, A simple method for reusing Western blots on PVDF membranes, BioTechniques 17:268-269.

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using a calculator to crunch the numbers. Here we present an approach that neatly overcomes many of these difficulties. Rather than writing the program as a macro for Microsoft Excel, we write it as a “macro” for WWW browsers using the JavaScript™ scripting language.

As noted in earlier Internet On-Ramp articles (2,3), JavaScript programs run right within a Web browser (Netscape Navigator or, theoretically, Internet Explorer; both of which are now free). You locate a program just by going to its Web site and run it right on the Web page. To use it off-line or to give a copy to friends, download it by saving the Web page. There is no installation; just opening the page in your browser makes it ready to run. And since it uses standard Web-page interaction elements like text areas and buttons, the interface should be familiar.

JavaScript Calculators

JavaScript is ideal for making specialty calculators. The programs themselves are fairly simple and small because the difficult work of drawing the user interface is handled by the browser. Thus the script is mostly concerned with the formulas. Here we present two master mix calculators that take different approaches.

Figure 1 shows a JavaScript program with an interface that is vaguely similar to the Excel macro (1). This page can be found at http://128.120.169.130/Fairclough/PCRCalc.html. Input concentrations, reaction size, number of tubes, etc. in the upper left corner and click on Calculate; the volumes of each reagent to include in the master mix appear on the right-hand side. The PRINT button creates a worksheet for your notebook.

The example in Figure 2 (http://www.attotron.com/pub/pcrtitr.htm) implements the “AmpliGrease” hot-start protocol (4) using PCR-compatible Cresol Red loading dye (5). However, many options can be changed to implement a variety of experimental protocols, and it does not necessarily need to be used only for hot start.

The program can calculate reagent volumes for a single reaction (by entering 1 row and 1 column), for one-dimensional titrations or for two-dimensional “box” titrations in which separate ingredients are titrated in the top and bottom mixtures. While the default distribution separates the ingredients according to the protocol described (4), reagents can be reassigned to “top” or “bottom” mixture, or split proportionately between them. To use stock solutions at different concentrations, type over the values in the boxes. A reagent can be omitted by entering a final concentration of zero. You can permanently reset the default values in your own copy of the program by editing the source code; default concentrations are set at the beginning of the program to make them easy to find. Clicking on Done puts the program in action; it asks you for the remaining concentrations if you are doing a titration, and it calculates a worksheet describing how much of each reagent to use in each tube for your experiment, complete with a “fudge factor” to take into account pipetting errors. The program checks your inputs to be sure they meet certain criteria (you must have at least one column, you can only titrate one ingredient in the top mixture and/or one in the bottom, etc.), and it won’t let you proceed until it is satisfied. But it will not necessarily complain about bizarre experimental designs. As with all JavaScript programs, the source code can be examined using your browser’s View Source option (View: Page Source in Navigator 4). You can use this to learn how to create your own specialized calculator or (with due credit to the authors!) modify the code to fit your needs.

REFERENCES