Technical Report

Spreadsheet Macro for Setting Up PCR Assay Tubes

BioTechniques 20:1088-1089 (June 1996)

Raymond P. Stowe and Duane L. Pierson¹

KRUG Life Sciences; ¹National Aeronautics and Space Administration, Lyndon B. Johnson Space Center, Houston, TX, USA

ABSTRACT

This article describes a Microsoft[®] Excel[®] macro designed to calculate PCR master mix amounts based on variations in the DNA template amounts added to each tube, the total number of PCR assay tubes being set up or both. This macro uses a dynamic dialog box to quickly calculate and display the new component volumes after changes in one or both of the variables. The PCR assay mix protocol can then be printed in a format suitable for record keeping. This macro was designed for use with the Windows[®] version of Excel but will also run on Macintosh[®] computers.

INTRODUCTION

The polymerase chain reaction (PCR) is a powerful tool in molecular biology research. PCR is a simple automated procedure that can be used to amplify (1) or modify a specific target DNA sequence. The reaction often is carried out in 25-, 50- or 100- μ L volumes contained in a 0.2-mL reaction tube. The final reaction mixture typically consists of the DNA target (template), four deoxynucleoside triphosphates (dATP, dCTP, dGTP and dTTP), complementary primers, Tris buffer and DNA polymerase. Some protocols also call for other reagents such as dimethyl sulfoxide (DMSO), glycerol or formamide to be included.

PCR protocols generally use master mix tubes. Individually dispensing the PCR reagents to each PCR assay tube is labor-intensive, uses large numbers of pipet tips and may introduce pipetting errors when small volumes are transferred. The use of master mix tubes is less labor-intensive and more cost-effective because fewer pipettings are made, and they also allow larger volumes to be pipetted into assay tubes, thus reducing pipetting errors inherent in transferring small volumes.

Computer programs exist that select optimized PCR primer pairs from DNA sequence files and establish optimal experimental conditions. However, calculations for determining the volumes of PCR components are frequently performed on handheld calculators. This approach is time-con-

suming and may lead to calculation errors. We present a program that has the advantages of minimal computer requirements and ease of calculations and record keeping. It was developed for use on the IBM[®] PC with Excel[™] for Windows[™] 4.0 (Microsoft, Redmond, WA, USA) and has been used in our laboratory for the past several months. The macros also can be run on the Macintosh[®] version of Excel.

The macro is contained in a file called **pcrmac.xlm**, which is loaded into the xstart directory within Excel. When Excel is started, a blank screen appears on the monitor instead of the usual unmodified spreadsheet (due to the **Hide** command built into the macro). An important note is that all active (displayed) worksheets must be closed for the macro to run properly. When the macro is started by the command key <CTRL/A>, a dialog box appears (Figure 1). The user can input the **Reaction Mixture Volume** by clicking on the appropriate button (25 or 100 μ L); the number of **Assay Tubes** (#) and the **Template** amount (μ L per reaction tube) are entered from the keypad (entry is not blocked in component columns, but they are not designed as data entry spaces). Clicking on the **OK** button automatically calculates the amounts of each assay component to be added to **Mix-Tube 1** or **Mix-Tube 2**. The amounts of **Mix-Tube 1** and **Mix-Tube 2** to be added to the reaction tubes are presented at the bottom of each column (**Volume [μ L/tube]**). The protocol can be printed to obtain a hard copy suitable as a notebook record (Figure 2). The user exits the dialog box by clicking on the **Cancel** button, which automatically hides the file. Quitting Excel produces a dialog

Component	Mix-Tube 1	Mix-Tube 2
10 x TRIS	25.00	
DMSO	12.50	
H2O	37.50	
dNTP (1.4)		20.00
H2O		105.00
Primer 1		10.00
Primer 2		10.00
Tag		10.00
Volume [μ L/tube]	7.50	15.50

Figure 1. The dialog box for the PCR assay mix protocol.

PCR ASSAY PROTOCOL			
Component	1 Assay (ul)	# Assays (ul)	
10X Buffer	2.5	25.00	
DMSO	1.25	12.50	
dNTP	12.5	125	
Template	2	20	
Primer 1	1	10	
Primer 2	1	10	
Taq	1	10	
	21.25	212.5	
H2O	3.75	37.5	
	25	250	

Mix 1			
10x buffer	25.00		
DMSO	12.50		
H2O	37.50		
	75		
Div/by	10	7.50	ul/assay

Mix 2			
dNTP (1:4)	20.00		
H2O	105.00		
	125		
Primers 1	10.00		
2	10.00		
Taq	10.00		
	155		
Div/by	10	15.50	ul/assay

Figure 2. The printout of the PCR assay mix components in table form.

box that asks the user if the changes in the macro file are to be saved.

The Macintosh version (**PCRMACInt.XLM**) of the macro can be opened by simply double-clicking on the icon. The **Hide** window functions have been removed, since Macintosh Excel does not have an "xlstart" directory. Upon opening, a minimized window will appear. The macro is started by the command key <CTRL/A>, and then by following the instructions above. The macro can be exited by clicking on the close square in the upper left-hand corner.

The assay setup displayed in Figure 2 is based on a method currently used in our laboratory. The PCR reagents are dispensed into the two mix tubes in the following manner: **Mix-Tube 1** contains 10x Tris buffer (Perkin-Elmer, Norwalk, CT, USA), DMSO (5% final conc.) and H₂O (to adjust the final volume); **Mix-Tube 2** contains dNTPs, both primers, *Taq* DNA polymerase (0.625 U/25 µL per assay tube) and water to bring reagents to correct concentrations. We only included 25- and 100-µL buttons, since the user could multiply for a 50-µL reaction volume. The macro can be modified to add/delete reagents as necessary and combine reagents (without *Taq* or DMSO) into a single mix tube for freezing. Increasing flexibility can also be performed by adjusting reagent (e.g., primers) concentrations to support fixed volumes in the macro. This division (Mix-Tube 1 and 2) is arbitrary so that this may also be applied to other configurations (e.g., top and bottom mixes). Additionally, by entering a larger number of tubes than necessary in the **Reaction Mixture Volume**, a "safety factor" can be figured in so that no shortage of reaction mixture occurs.

MODIFYING THE MACRO

The macro file (**pcrmac.xlm**) is formatted as an interna-

tional macro; the sheet is "hidden" to prevent accidental modification. To view and edit the macro sheet, choose **Unhide** from the **File** menu. After viewing/modifying the macro, choose the **Hide** command to protect against accidental error when running the macro again. The Macintosh version (**PCRMACInt.XLM**) does not require the **Unhide** command. The window can be maximized to view the program codes. The user can then minimize the window and save the changes before exiting the macro.

OBTAINING THE MACRO

The software discussed in this paper is available for downloading from the BioComputing section of the *BioTechniques* World Wide Web site located at www/BioTechniques.com.

ACKNOWLEDGMENTS

We thank Christine Wogan for her editorial work. This work was supported by NASA Grant No. NAS-18492.

REFERENCE

1. Innis, M.A., D.H. Gelfand, J.J. Sninsky and T.J. White. 1990. PCR Protocols: A Guide to Methods and Applications, p. 3-12. Academic Press, New York.

Received 18 January 1996; accepted 5 April 1996.

Address correspondence to:

Raymond P. Stowe
KRUG Life Sciences, Inc.
 1290 Hercules
 Suite 120
 Houston, TX 77058, USA