Low-Volume 384-Well PCR Set-Up Using Promega's PCR Master Mix and the TTP LabTech mosquito™ Liquid Handling System

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OVERVIEW

Here we demonstrate the robust amplification of genomic DNA targets in a 2ul total volume PCR reaction. The TTP LabTech mosquito™ was used for delivery of DNA sample, PCR primers, and amplification master mix. A MJ Research DNA Engine® (PTC-200™) Peltier Thermal Cycler with a 384-well sample block was used to amplify genomic DNA targets of interest. Agarose gel analysis shows the robust amplification of all targets in this 2ul total volume reaction. DNA titration experiments show successful amplification down to 2 picograms of human genomic DNA.

INTRODUCTION

PCR amplification is a powerful tool that has applications in a variety of areas including gene expression analysis and DNA sequencing. The miniaturization of PCR assays allows the researcher to increase throughput and reduce reagent cost. High-precision liquid handling combined with robust, scalable reagent chemistries, can bring PCR to the higher density formats required to increase throughput of today's genomic assays.

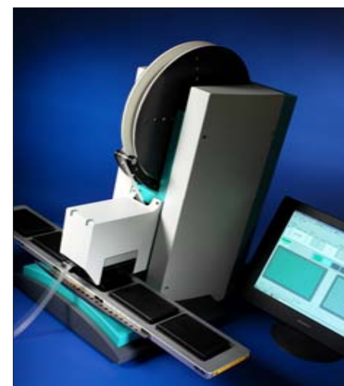
Promega's PCR Master Mix[(a)] is a premixed, ready-to-use solution containing *Taq* DNA Polymerase, dNTPs, $MgCl_2$ and reaction buffers at optimal concentrations for efficient amplification of DNA templates by PCR. The Master Mix is supplied at a 2X solution, making PCR set-up fast and convenient. Master Mix is added 1:1 to the total volume of amplification primers and template DNA in the reaction. This simple reaction set-up makes the use of PCR Master Mix highly conducive to automation.

The scalability of PCR using Promega's PCR Master Mix is demonstrated on the TTP LabTech mosquito™ liquid handling system. DNA amplification by PCR was performed in 2ul total volume reactions. The MJ Research DNA Engine® (PTC-200™) Peltier Thermal Cycler with a 384-well sample block was used for amplification. To test the precision and accuracy of dispensing PCR components in a 384-well amplification plate, a checkerboard experiment was performed to examine cross-contamination and multiple target amplification. In addition, sensitivity of PCR amplification using Promega's 2X PCR Master Mix was tested by performing a template DNA titration experiment with the mosquito™.

METHODS

Figure 1. Promega's PCR Master Mix[(a)]. Promega's PCR Master Mix is made up of a 2X ready-to-use solution containing optimal concentrations of reaction components for efficient amplification of DNA templates by PCR. 2X PCR Master Mix contains the following components: 50units/ml of *Taq* DNA Polymerase supplied in a proprietary reaction buffer (pH 8.5), 400uM dATP, 400uM dGTP, 400uM dTTP, 400uM dCTP, and 3mM $MgCl_2$. As demonstrated in Figure 1, PCR Master Mix is added 1:1 to the total volume of primers, template and water contained within the reaction vessel.

A.



B.

- •Disposable tips for zero cross-contamination
- •Positive displacement reagent dispensing from 50nl to 1.2µl
- •No tip washing required
- •Bandolier of 26,000 or 36,000 pipettes per reel of mosquito tape
- •CVs less than 10%
- •Two to five deck positions for 384 or 1536-well plates
- •Small footprint for bench top use
- •User-friendly programming software

Figure 2. TTP LabTech mosquito™ hardware configuration. A) The TTP LabTech mosquito™ Liquid Handling System. B) Features of the Equator™ system that were useful for this application. Additional technical details can be obtained from TTP LabTech.

LOW-VOLUME DISPENSING

Step	Volume	Location	Final concentration
1. Add 2X PCR Master Mix	1000nl	Columns 1-24	1X
2. Add Factor V primer mix	500nl	Columns 1-6	2pmol
3. Add Factor II primer mix	500nl	Columns 7-12	2pmol
4. Add HFE H63D primer mix	500nl	Columns 13-18	2pmol
5. Add HFE C282Y primer mix	500nl	Columns 19-24	2pmol
6. Add genomic DNA*	500nl	Odd columns	2ng
7. Add genomic DNA**	500nl	Even columns	2ng

Table 1. Multiple target amplification and cross-contamination analysis. The mosquito™ was programmed to add PCR Master Mix, primers, and genomic DNA by column to a 384-well MJ Research PCR cycle plate in the steps listed above such that four different products could be amplified from the same human genomic DNA.

Factor V and Factor II are two different clotting factors involved in the blood clotting cascade. HFE is the hemochromatosis gene, associated with iron storage throughout the body. HFE H63D primers amplify a segment of exon 2 in the HFE gene. HFE C282Y primers amplify a segment of exon 4 in the HFE gene.

To test for cross-contamination during PCR set-up, a checker board experiment was performed such that DNA was added to every other well in each column of the 384-well PCR plate. * denotes odd numbered columns that received DNA in rows A, C, E, G, I, K, M, and O. ** denotes even numbered columns that received DNA in rows B, D, F, H, J, L, N, and P. 500nl of water was added to wells receiving no DNA.

Following addition of the PCR components, each reaction well was manually overlaid with 10ul of mineral oil and placed in the thermal cycler. Cycling conditions were as follows: 94C, 1:30 x 1 cycle; 94C, 0:30, 60C, 0:30, 72C, 0:30 x 35 cycles; 72C, 1:30 x 1 cycle; 4C soak.

Step	Volume	Location	Final concentration
1. Add 2X PCR Master Mix	1000nl	Columns 1-7	1X
2. Add HFE C282Y primer mix	500nl	Columns 1-7	2pmol
3. Add human genomic DNA*	500nl	Column 1	1 - 0.01ng
	400nl	Column 2	0.8-0.008ng
	300nl	Column 3	0.6-0.006ng
	200nl	Column 4	0.4-0.004ng
	100nl	Column 5	0.2-0.002ng
	50nl	Column 6	0.1-0.001ng
	0nl	Column 7	0ng
4. Add water to final 2ul volume	100nl	Column 2	
	200nl	Column 3	
	300nl	Column 4	
	400nl	Column 5	
	450nl	Column 6	
	500nl	Column 7	

Table 2. DNA titration. The mosquito™ was programmed to add PCR Master Mix, C282Y primers, water, and different volumes of human genomic DNA to achieve a template titration across the 384-well PCR plate.

Three different stock solutions of human genomic DNA were used to achieve the range of final DNA concentrations noted on the chart above. A 2ng/ul DNA stock solution was added to rows A-D of a 384-well Greiner reagent plate. Final DNA concentrations from this stock solution ranged from 1ng/2ul reaction down to 0.1ng/2ul reaction. A 0.2ng/ul DNA stock solution was added to rows E-H of the reagent plate. Final DNA concentration from this stock ranged from 0.1ng/2ul reaction down to 0.01ng/2ul reaction. A third 0.02ng/ul DNA stock solution was added to rows I-L of the reagent plate. Final DNA concentration from this stock ranged from 0.01ng/2ul reaction to 0.001ng/2ul reaction.

Following the dispensing of all PCR components, each reaction well was manually overlaid with 10ul of mineral oil and placed in the thermal cycler. The same cycling conditions were used as described for Table 1.

RESULTS

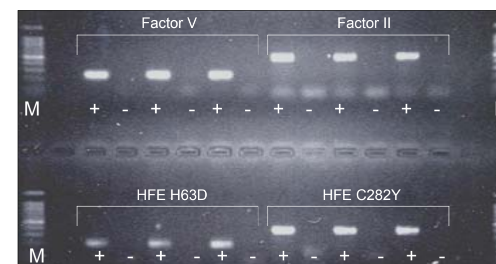


Figure 3. Target amplification and cross-contamination results. The amplification of the Factor V, Factor II, HFE H63D, and HFE C282Y targets was successful. The expected size for the Factor V product is 254bp, Factor II – 500bp, HFE H63D – 208bp, and HFE C282Y – 390bp.

To analyze amplification success, the entire 2ul PCR reaction volume was combined with blue/orange loading dye and run on a 2% agarose gel for 25 minutes at 100 volts. The gel was stained in an ethidium bromide solution for 4 minutes, then destained in water for 15 minutes.

M = 100bp DNA ladder, + = DNA added to well, - = no DNA added to well.

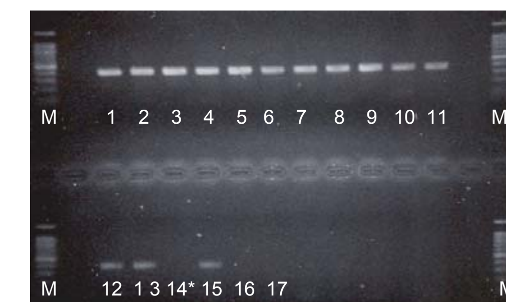


Figure 4. DNA titration results. The titration of human genomic DNA into the 2ul PCR amplifications was successful. Results show a decrease in amplification of HFE C282Y target with the addition of smaller amounts of DNA. The amplification of C282Y using as little as 2pg of human genomic DNA could be detected with gel electrophoresis. The same electrophoresis conditions were used as described in the legend for Figure 3.

M = 100bp DNA ladder, 1 = 1ng DNA, 2 = 0.8ng DNA, 3 = 0.6ng DNA, 4 = 0.4ng DNA, 5 = 0.2ng DNA, 6 = 0.1ng DNA, 7 = 80pg DNA, 8 = 60pg DNA, 9 = 40pg DNA, 10 = 20pg DNA, 11 = 10pg DNA, 12 = 8pg DNA, 13 = 6pg DNA, *14 = 4pg DNA (reaction misloaded onto gel), 15 = 2pg DNA, 16 = 1pg DNA, 17 = 0 DNA (negative control).

CONCLUSIONS

1. Promega's PCR Master Mix is scalable down to a 2ul total volume PCR amplification as demonstrated by robust amplification of Factor V, Factor II, HFE H63D and HFE C282Y human genomic DNA targets.
2. Promega's PCR Master Mix is sensitive as demonstrated by detectable amplification of 2pg of human genomic DNA in this application.
3. The TTP LabTech mosquito™ platform is a user friendly dispensing system for performing low volume PCR set-up.
4. The TTP LabTech mosquito™ dispenses PCR components without cross-contaminating from well to well.
5. The TTP LabTech mosquito™ can be used to optimize low-volume PCR amplifications.

REFERENCES

1. Promega Product Information sheet # 9PIM750
2. http://www.ttplabtech.com/mosquito/mosquito.htm

PATENTS / DISCLAIMERS

(a)The PCR process, which is the subject of European Pat. Nos. 201,184 and 200,362 and U.S. Pat. Nos. 4,683,195, 4,965,188 and 4,683,202 owned by Hoffmann-LaRoche*, is covered by patents issued and applicable in certain countries. Promega does not encourage or support the unauthorized or unlicensed use of the PCR process. Use of this product is recommended for persons that either have a license to perform PCR or are not required to obtain a license. *In the U.S., effective March 29, 2005, the above primary U.S. Pat. Nos. 4,683,195, 4,965,188 and 4,683,202 will expire. In Europe, effective March 28, 2006, the above primary European Pat. Nos. 201,184 and 200,362 will expire.

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