Oct., 2013

Consistent serial dilution results using EzMate™ 401 Automated Pipetting System with 8-channel APM

Introduction

The highly accurate and precise EzMate™ Automated Pipetting System is specialized in handling low-volume pipetting tasks. The highly uniform 8-channel Automated Pipetting Module (APM) makes it an ideal tool for fast, accurate pipetting. In this application note, we demonstrated a consistent serial dilution operation using EzMate™ 401 Automated Pipetting System.

Material

Equipment: Arise EzMate™ 401 with 8-channel 50 µl Automated Pipetting Module (APM50-8)
Roche LightCycler® 480 Real-Time PCR System

Reagent: Finnzymes DyNAmo™ SYBR Green qPCR Kit, # F-415-L

Template: 101 bp synthetic DNA with following sequence, 1X working Conc. 1 pM:
AAC TTG GCT TTA ATG GAC CTC CAA TTT TGA GTG TGC ACA AGC TAT AGA ACA CCA CGT AAG ACA TAA AAC GGC CAC ATA TGG TGC CAT GTA AGG ATG AAT GT

Primers: Forward: AAC TTG GCT TTA ATG GAC CTC CA, working Conc. 300 nM
Reverse: ACA TTC ATC CTT ACA TGG CAC CA, working Conc. 300 nM

Consumables: Roche LightCycler® 480 Multiwell Plates 384, # 047729749001
Axygen® 50 ul Robotic Tip, w/o filter, Non-Sterile, # FX-50-R

Methods

1. Prepare 3 sets of 4 fold serial dilution separately, starting from 1 pM DNA. (figure 1.)

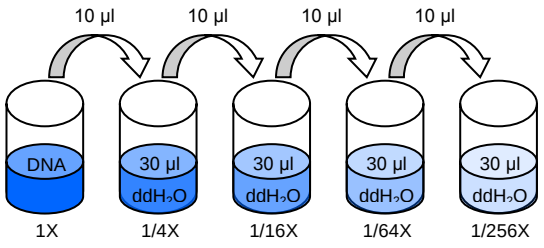

Figure 1

P.1, continued…

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2. Prepare the pre-mix solution (listed in the table 1 below). Use EzMate™ 401 with 8-channel 50 µl Automated Pipetting Module (APM50-8) to dispense 18 µl/well of the premix solution into each well in a clean 384-well qPCR plate.

Table 1. Pre-mix solution

	µl/well	Total volume (µl) *
ddH2O	6.4	3014
MasterMix	10	4850
ROX (Optional)	0.4	194
Primer-F	0.6	291
Primer-R	0.6	291
Total	18	8730

* includes dead volume and extra volume

3. Use EzMate™ 401 with APM50-8 to dispense 2 µl of the diluted DNA into each well of the 384-well plate (blue frames in figure 2 below for dilution series 1, red frames for dilution series 2 and green frames for dilution series 3), triplicate 8-well for each concentration.

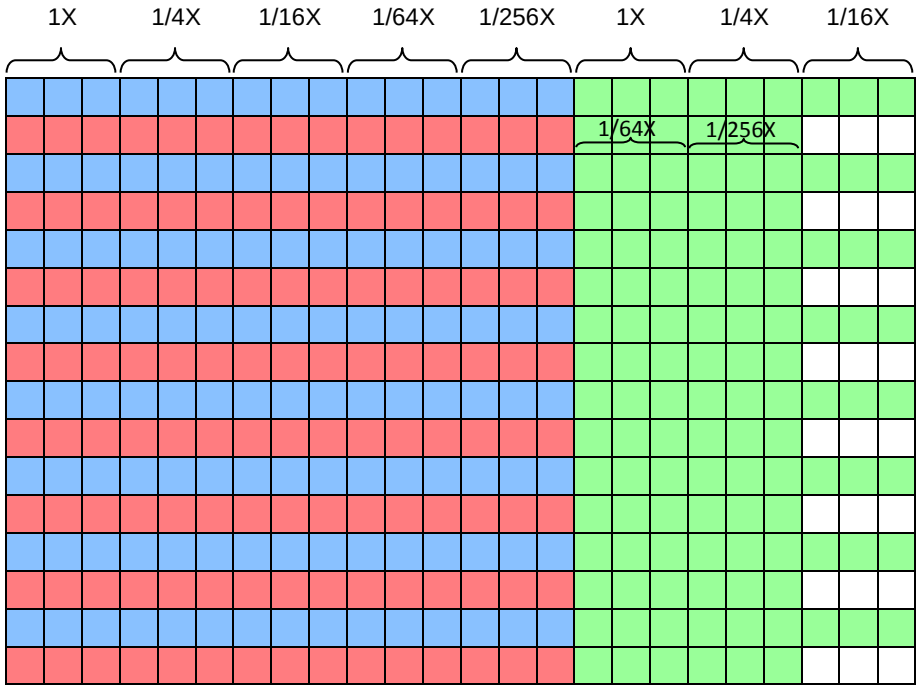

Figure 2

P.2, continued…

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4. After dispensing, seal the plate, gently tap its side and then centrifuge it to remove any bubbles in the well. Run the qPCR experiment under the following cycling conditions: (below, table 2)

Table 2. Cycling conditions

Initial denaturation	95°C	10 min
Cycling 40 X	95°C	10 sec
	60°C	15 sec
	reading	

Result

1. qPCR results

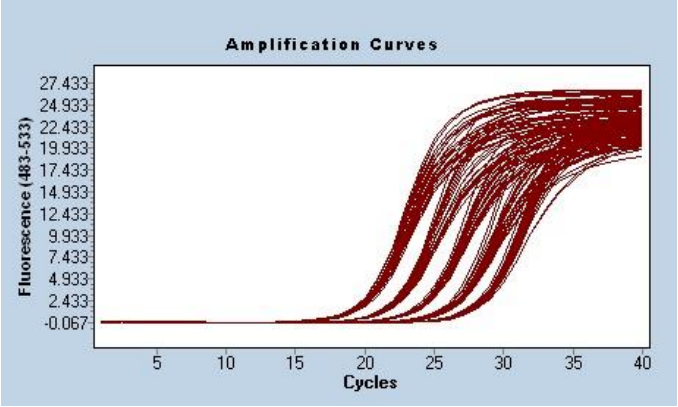

Figure 3. Amplification curve of Roche LightCycler® 480

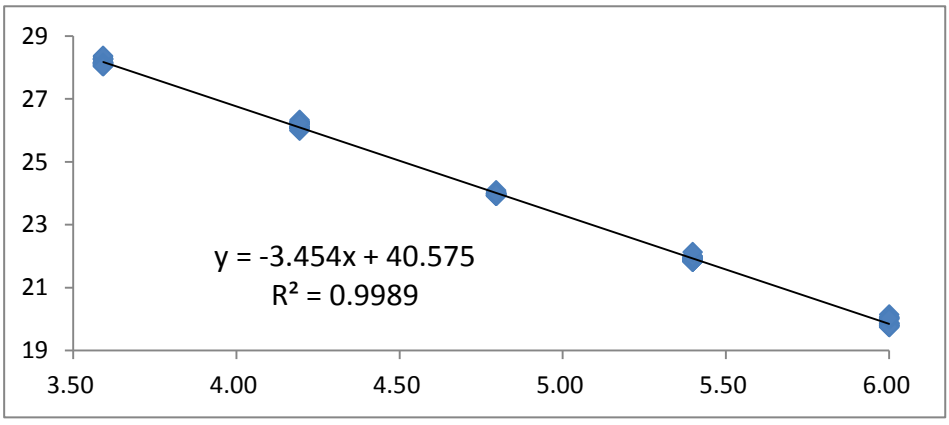

Figure 4. Standard Curve for all dilutions

PCR efficiency = 94.77%

P.3, continued…

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	Log Conc.	Mean Cp	STD Cp
Dilution Series 1	6.00	19.80724827	0.044662008
	5.40	21.86468467	0.042291606
	4.80	23.95229878	0.041827036
	4.19	26.12377573	0.102822209
	3.59	28.16375531	0.087791064

$y = -3.4834x + 40.688$ $R^2 = 0.9994$

	Log Conc.	Mean Cp	STD Cp
Dilution Series 2	6.00	19.85012988	0.038451248
	5.40	21.93548871	0.021858968
	4.80	23.99964799	0.033846217
	4.19	26.19269906	0.122907813
	3.59	28.17248559	0.05113412

$y = -3.4717x + 40.680$ $R^2 = 0.9994$

	Log Conc.	Mean Cp	STD Cp
Dilution Series 3	6.00	20.04255477	0.044660059
	5.40	21.96408712	0.083236093
	4.80	23.98817673	0.041825192
	4.19	26.17952653	0.083630701
	3.59	28.15561896	0.126325386

$y = -3.3953x + 40.349$ $R^2 = 0.9989$

2. The standard deviations for the Cps for each concentration replicates are less than 0.5.
3. The R^2 values for all the standard curves are greater than 0.98.
4. The PCR efficiencies are all within 100% ± 10%.

Conclusion

The high accuracy and precision of the EzMate™ automated pipetting system in µl-level liquid pipetting combined with the uniform 8-channel Automated Pipetting Module make it an ideal tool for fast and consistent pipetting task. The application-oriented EzStarter™ control software is specially designed to meet modern day molecular biology laboratories' need.