

Evaluate the Performance of Real-time PCR Instruments by Using EzMate™ 401 Automated Pipetting System

Introduction

The highly accurate and precise EzMate™ 401 Automated Pipetting System is specialized in handling low-volume pipetting tasks, such as special qPCR experiments, which can be used to evaluate the realtime PCR instrument's performance. In this application note, we use EzMate™ 401 to prepare a special qPCR experiment to evaluate the well-to-well variation of a real-time PCR instrument.

Material

Equipment: Arise EzMate™ 401 with APM50-1

Roche LightCycler® 480 Real-Time PCR System

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

Template: 101bp synthetic DNA with following sequence,

1X working Conc. 1pM:

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. 300nM

Reverse: ACA TTC ATC CTT ACA TGG CAC CA, working Conc. 300nM

Consumables: Roche LightCycler® 480 Multiwell Plates 384, # 047729749001

Axygen 50ul Robotic Tip, w/o filter, Non-Sterile, # FX-50-R

Methods

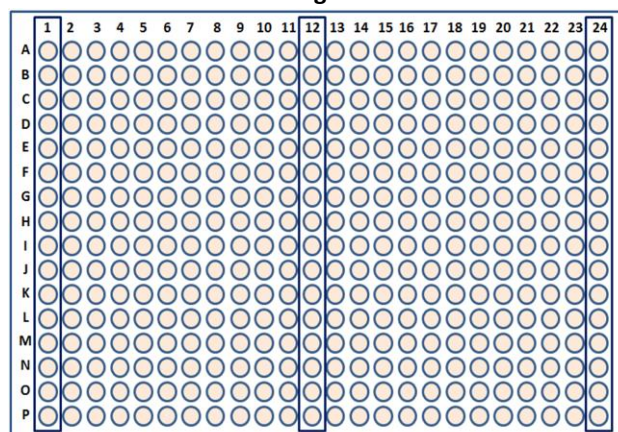
Prepare the pre-mix solution (listed in the table 1. below) in a 1.5 ml tube, and invert the tube several times to mix the content thoroughly (do not vortex the tube).

Table 1

	µl/well	60 well (µl)
ddH ₂ O	6.4	384
MasterMix	10	600
ROX (Optional)	0.4	24
Primer-F	0.6	36
Primer-R	0.6	36
Template	2	120
Total	20	1200

Use EzMate™ 401 to dispense 20 µl/well of the premix solution into each well in columns 1, 12 and 24 of a clean 384-well qPCR plate (blue frames in figure 1. below).

Figure 1



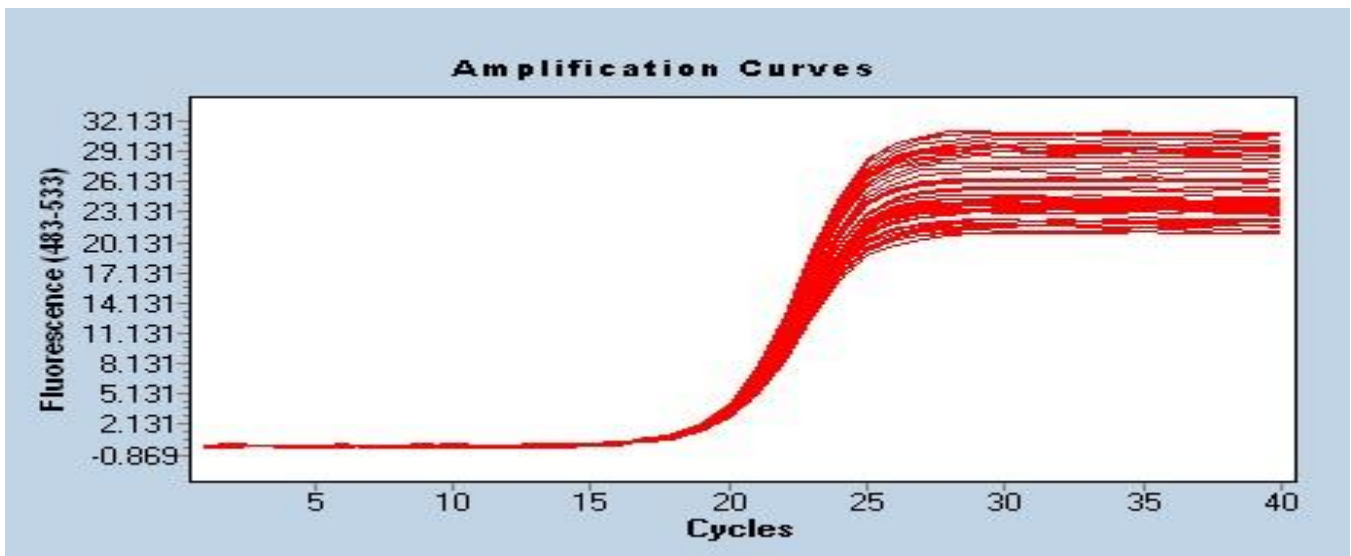
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After dispensing, seal the plate, gently tap its side and then centrifuge it to remove any bubbles in the well. Run the qPCR experiment under the following cycling conditions: (below, table 2.)

Table 2

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

Result



Cp mean = 19.47316854

Cp SD = 0.031756082

Acceptable criteria: SD of Cp should be ≤ 0.5.

The test result indicates that the tested instrument performs well. The variations between wells are very small.

Conclusion

The high accuracy and precision of the EzMate™ automated pipetting system in µl-level liquid transfers make it a suitable tool to prepare special qPCR experiments for the performance evaluation of any real-time PCR instrument. By using the **synthetic oligonucleotide** as the target template, users can eliminate potential problems caused by the uncertainty of the natural DNA sample, and improve the reproducibility of the evaluation.