Complete Automation of Quantitative Polymerase Chain Reaction Assays on the Agilent Bravo Automated Liquid Handling Platform

Application Note

Authors

David Knorr
Manuel Gomez
Stephen Lappin
Agilent Technologies, Inc.
Santa Clara, CA USA

Abstract

The Agilent Bravo Automated Liquid Handling Platform has been shown to be an effective way to automate setup of a variety of quantitative polymerase chain reaction (QPCR) assays. QPCR reactions to examine a variety of housekeeping genes from cDNAs derived from different human tissues were set up on the Bravo Automated Liquid Handling Platform and by hand. Dilutions performed by each method showed similar slopes and good linearity and targets amplified by either method also gave similar Ct values. When examining cDNAs from fetal and adult lung, differential expression was observed in the B2M whereas GUSB and TBP remained similar. The Bravo Automated Liquid Handling Platform should be useful to scientists interested in speeding up setup while maintaining reliability when performing QPCR studies.

Introduction

QPCR assays have become widespread for detecting and measuring the abundance of specific genetic targets. Technologies developed to detect and monitor PCR product formation and accumulation include TaqMan and Molecular Beacons, which measure accumulation of PCR product-specific fluorescent probes. Another method involves measuring the increase of fluorescent DNA intercalating dyes such as SYBR Green. Agilent Technologies provides tools and reagents to speed reaction set-up while reducing possible mistakes and inaccuracies that may arise from performing these steps manually. Here we describe using the Bravo Automated Liquid Handling Platform to set up a series of SYBR Green QPCR experiments to examine relative gene expression levels for a number of targets in several tissues.

The Bravo Automated Liquid Handling Platform has nine plate deck positions, which along with an Agilent BenchCel Workstation and an Agilent Microplate Centrifuge can fully automate setup of the QPCR protocols without manual intervention. Described below is a protocol, written for a workstation containing the Bravo, a BenchCel Microplate Handling Workstation and an Agilent PlateLoc Thermal Microplate Sealer to automatically handle reagent dilution, aliquoting, and final assay plate preparation.

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Experimental

First Strand cDNA mixtures derived from poly[A] + total RNA from Human adult and fetal lung tissue, adult Heart and HeLa cells (each at 500 pg/µL) were used as templates. A set of ten Human housekeeping gene primers was obtained from RealTimePrimers (product number HHK-1). An additional set of primers specific for ß-Actin (included with Stratagene cDNAs) was also used. Final reactions were prepared by combining 250 pg of cDNA template diluted to 10 µL in water with a combination of one of the specific primer pairs and master mix (Stratagene Brilliant II SYBR Green QPCR Master Mix, Agilent Part Number 600828) diluted to 15 µL. Each cDNA / target reaction was prepared in duplicate. Identical reaction mixes were prepared either by hand or using the Agilent Bravo according to the Stratagene recommendations. Reaction plates were sealed automatically with a clear QPCR-compatible seal (Agilent Part Number 17318-001) using an Agilent PlateLoc Thermal Microplate Sealer. Reactions were developed using a Stratagene Mx3005P Real Time Thermal Cycler (G5105A) for 40 cycles according to the kit recommendations. Data was collected and processed using MxPro v4.10 software.

Results and Discussion

To compare possible differences between using a Bravo and hand-pipetting, cDNA from fetal lung tissue was diluted 1:2 seven times to provide template concentrations from 500 – 7.8 pg/µL. Linear regression analysis of the Ct values plotted against template starting concentration gave R^2 values of 0.973 for the Bravo and 0.983 for the hand-pipetted sets.

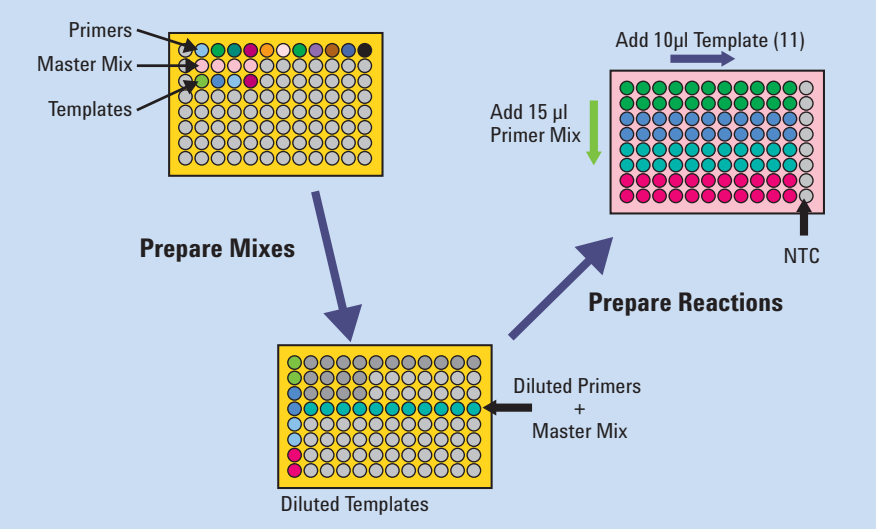

Figure 1. General workflow used to prepare QPCR reactions.

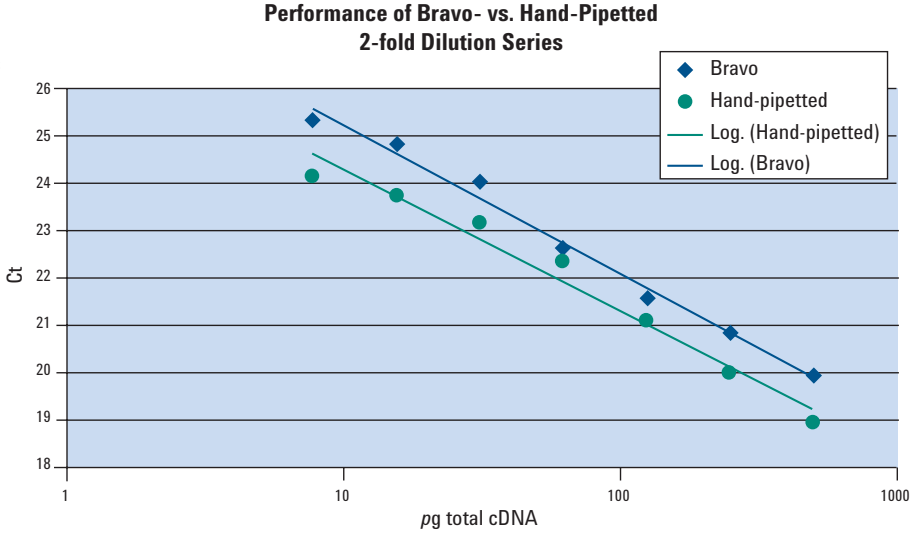

Figure 2. Performance of Bravo vs. hand-pipetted 2-fold dilutions

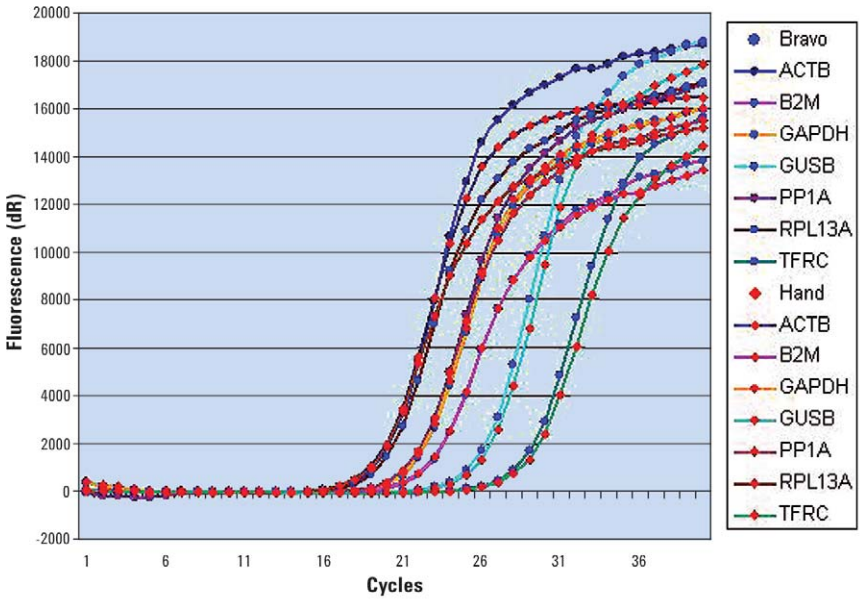

Figure 3. Amplification plots for seven different housekeeping genes prepared using the Bravo, or by hand in amplifying targets from fetal lung tissue. Target identities are given in the legend.

Differences between Agilent Bravo and hand-pipetting were also examined by looking at a number of different housekeeping genes. The plot shown below shows each plot line as an average between replicate wells for both the relative differences of different starting concentrations for different gene targets.

The table below gives the absolute differences in Ct values between targets that were prepared by the Bravo and by hand. Most targets gave very similar Ct values, indicating similar relative abundance for each target.

Comparison of Ct values obtained When Preparing Targets using the Bravo or by Hand

Target	Fetal Lung			HeLa			Fetal Heart		
	Bravo	Hand	Δ	Bravo	Hand	Δ	Bravo	Hand	Δ
ACTB	20.37	20.04	0.33	19.87	20.09	0.22	19.52	19.54	0.02
B2M	23.86	23.6	0.26	22.64	22.93	0.29	20.21	20.15	0.06
GAPDH	22.74	22.44	0.3	19.28	19.36	0.08	18.6	18.43	0.17
GUSB	26.46	26.64	0.18	25.12	25.18	0.06	25.32	25.27	0.05
HPRT1	29.77	29.91	0.14	25.86	26.33	0.47	25.92	25.79	0.13
PGK	26.47	26.35	0.12	22.39	22.61	0.22	22.77	22.62	0.15
PP1A	22.53	22.28	0.25	20.94	21.37	0.43	20.5	20.44	0.06
RPL13A	20.67	20.13	0.54	20.21	20.36	0.15	18.77	18.55	0.22
TBP	28.85	29.03	0.18	28.55	28.85	0.3	27.85	28.11	0.26
TFRC	29.51	29.71	0.2	24.66	24.91	0.25	27.28	27.4	0.12
ß-actin	20.78	20.83	0.05	20.53	20.84	0.31	17.52	17.75	0.23

The data show good correlation for each target whether developed using the Agilent Bravo or by hand.

Examples of conserved and differential gene expression are given in **Figure 4**. While genes for GUSB and TBP showed similar profiles and Ct values (around 23 and 25.5 respectively), the serum protein beta-2 microglobin (B2M) shows an apparent difference in expression level with the adult version showing an approximately 4-fold increase in expression over the fetal version.

Conclusion

The Bravo Automated Liquid Handling Platform, along with an Agilent BenchCel Workstation and Agilent Microplate Centrifuge, can be used to quickly automate QPCR assays without sacrificing data quality while greatly increasing overall throughput.

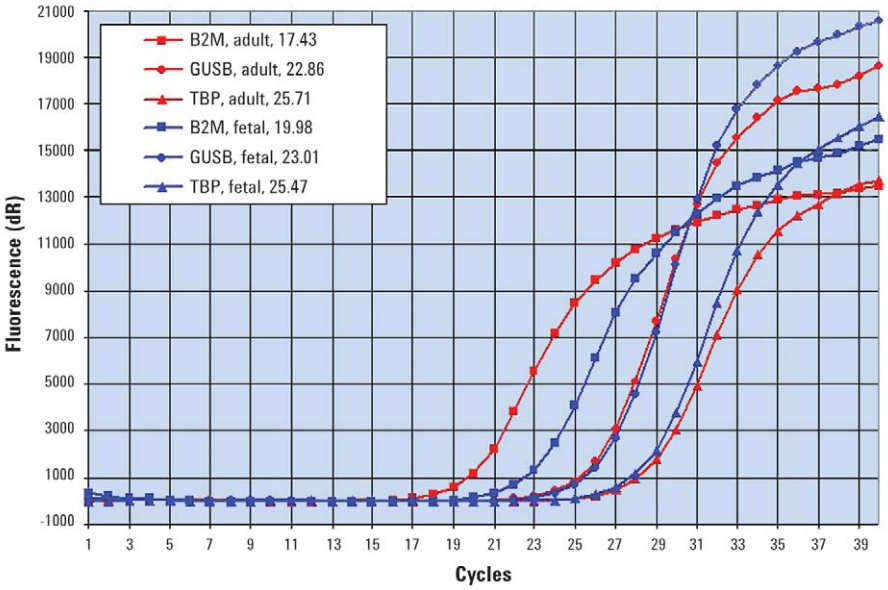

Figure 4. Amplification of three genes in fetal and adult lung. Ct values for each target are given in the legend.

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