Validation of VERSA 10 Workstation for Real-Time PCR Setup using cDNA from two Genes of Trout Fish

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I. Abstract

A major challenge in automated PCR technology is efficiently scaling up to high-throughput PCR using microlitre volumes of cDNA samples while also ensuring low cross-contamination and high accuracy and reproducibility. Aurora Biomed Inc. has recently launched its VERSA 10 PCR Setup Workstation (Figure 1). as an answer for accurate and reproducible high-throughput PCR. In this work, real-time PCR setups for 2 trout fish genes (SF10 and SF1a) were configured using Aurora Biomed's VERSA 10 Workstation to dispense 1 or 2 µL-volumes of cDNA template into an alternate column to check for cross-contamination. The statistical analysis from the replicates and inter-channel capabilities indicates that each of the channels reproducibly dispenses accurate volumes along the deck while conditions generate amplicons of the same size, making the VERSA 10 PCR Setup Workstation an excellent alternative for high-throughput and real-time PCR.

II. Introduction

PCR technology is commonly used in a variety of fields, including high-throughput research and diagnostics laboratories. To meet this need, both thermal cycling and the automation of liquid handling have evolved.[1-3] A major step in PCR automation has been to set up reactions with 1 to 2 µL template DNA thereby decreasing the risk of cross-contamination, while achieving accuracy and reproducibility. Aurora Biomed Inc. has recently launched the compact, multi-channel VERSA 10 PCR Setup Workstation. The validation data on this system is herein presented.

III. Objectives

1. To validate the VERSA 10 workstation for
   - Low cross-contamination
   - Accurate reagent dispensing
   - Accurate dilution of template DNA
   - Uniform temperature conditions
2. To assess the VERSA 10 for hands-free processing

IV. Materials & Methods

The validation of the VERSA 10 PCR Setup Workstation was conducted as follows:

1. **DNA template:** Amplicons of the following 2 trout fish genes were used as a template:
   a. S10: Ribosomal Protein S10
   b. SF1a: Steroidogenic Factor 1
2. **Master mixes and primer sets:** Two separate master mixes (primers in RNAse-free water and iTaq™ SYBR® Green Supermix With ROX; Cat #172-5853, Bio-Rad Laboratories, Mississauga, Ontario, Canada) were pipetted into a 96-well plate.
3. **Deck:** The deck was configured as shown in Figure 2.
4. **Reaction**: The 15 µL reaction mixture was set up by dispensing 1 or 2 µL of the aforementioned cDNA templates (plate map in Figure 3) on the VERSA 10 Workstation. The plate was manually transferred to the Stratagene Mx3000P thermal cycler. The MxPro Mx3000P (v4.01 Build 369, Schema 80 software; Stratagene Corp, La Jolla, USA) was used for data analysis.

Figure 1: VERSA 10 Workstation with HEPA / UV enclosure

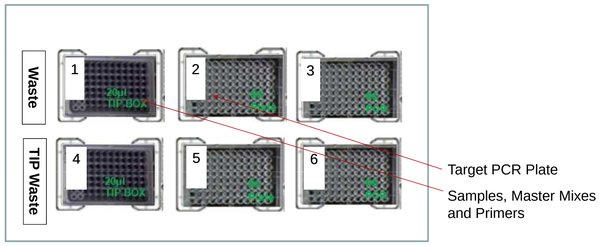

Figure 2: Deck layout of the VERSA 10 PCR Setup Workstation

Channel #	Plate Format	Replicates 1	2	3	4	5	6	7	8	9	10	11	12
1	A	S10 2ul	SF1a 2ul	S10 2ul	SF1a 2ul	S10 2ul	SF1a 2ul	S10 1ul	SF1a 1ul	S10 1ul	SF1a 1ul	S10 1ul	SF1a 1ul
2	B	S10 2ul	SF1a 2ul	S10 2ul	SF1a 2ul	S10 2ul	SF1a 2ul	S10 1ul	SF1a 1ul	S10 1ul	SF1a 1ul	S10 1ul	SF1a 1ul
3	C	S10 2ul	SF1a 2ul	S10 2ul	SF1a 2ul	S10 2ul	SF1a 2ul	S10 1ul	SF1a 1ul	S10 1ul	SF1a 1ul	S10 1ul	SF1a 1ul
4	D	S10 2ul	SF1a 2ul	S10 2ul	SF1a 2ul	S10 2ul	SF1a 2ul	S10 1ul	SF1a 1ul	S10 1ul	SF1a 1ul	S10 1ul	SF1a 1ul
5	E	S10 2ul	SF1a 2ul	S10 2ul	SF1a 2ul	S10 2ul	SF1a 2ul	S10 1ul	SF1a 1ul	S10 1ul	SF1a 1ul	S10 1ul	SF1a 1ul
6	F	S10 2ul	SF1a 2ul	S10 2ul	SF1a 2ul	S10 2ul	SF1a 2ul	S10 1ul	SF1a 1ul	S10 1ul	SF1a 1ul	S10 1ul	SF1a 1ul
7	G	S10 2ul	SF1a 2ul	S10 2ul	SF1a 2ul	S10 2ul	SF1a 2ul	S10 1ul	SF1a 1ul	S10 1ul	SF1a 1ul	S10 1ul	SF1a 1ul
8	H	S10 2ul	SF1a 2ul	S10 2ul	SF1a 2ul	S10 2ul	SF1a 2ul	S10 1ul	SF1a 1ul	S10 1ul	SF1a 1ul	S10 1ul	SF1a 1ul

Figure 3: Plate map of the PCR setup to check cross-contamination, inter-channel variability, and replication reproducibility.

Table 1: Thermocycling Conditions

Program:		Time (s)	Temp (°C)
		60	95
	40 x	20	95
		30	60
		40	72
		+ Dissociation curve	

V. Results

The amplification plots and dissociation curves of the replicates for both the S10 and SF1a genes are presented below for 1 µL and 2 µL of cDNA templates.

Table 2: Ct values (1 µL of S10 or SF1a cDNA) for gene amplification

Well 1	Rep 1	Well 2	Rep 2	Well 3	Rep 3	Mean Rep	SD Rep	CV% Rep
A7	19.01	A9	19.01	A11	18.86	18.96	0.09	0.46
B7	19.06	B9	18.91	B11	18.84	18.94	0.11	0.59
C7	19.22	C9	19.13	C11	19.32	19.22	0.10	0.49
D7	19.11	D9	19.02	D11	19.53	19.22	0.27	1.42
E7	19.01	E9	19.17	E11	18.52	18.90	0.34	1.79
F7	19.12	F9	19.06	F11	19.02	19.07	0.05	0.26
G7	19.12	G9	19.09	G11	19	19.07	0.06	0.33
H7	18.99	H9	19.17	H11	19.12	19.09	0.09	0.49
Mean	19.08		19.07		19.0263			
SD	0.07746		0.08928		0.30896			
Inter-channel % CV	0.40597		0.46818		1.62385			
A8	25.43	A10	25.71	A12	26.85	26.00	0.75	2.89
B8	25.51	B10	25.85	B12	26.69	26.02	0.61	2.33
C8	25.6	C10	25.91	C12	26.71	26.07	0.57	2.20
D8	25.66	D10	26.02	D12	26.84	26.17	0.60	2.31
E8	25.41	E10	25.95	E12	26	25.79	0.33	1.27
F8	25.48	F10	26.98	F12	25.63	26.03	0.83	3.17
G8	25.63	G10	25.84	G12	25.26	25.58	0.29	1.15
H8	25.73	H10	25.84	H12	25.25	25.61	0.31	1.23
Mean	25.5563		26.0125		26.1538			
SD	0.11563		0.40142		0.70368			
Inter-channel CV%	0.45244		1.54317		2.69056			

Replicates obtained from each channel in the amplification of the S10 gene (1 µL of cDNA) provide confidence of variability (CV%) values ranging from 0.26 to 1.79, indicating very precise liquid handling. Similarly, the CV% values among the 8 channels ranged from 0.4 to 1.62 suggesting a high degree of accuracy for all 8 channels of the workstation. Furthermore, low CV% values were obtained in the amplification of the SF1a gene where the replication and inter-channel CV% range was 1.15 to 3.17 and 0.45 to 2.69, respectively.

Table 3: Ct values (2 µL of S10 and SF1a cDNA) for gene amplification

Well 1	Rep 1	Well 2	Rep 2	Well 3	Rep 3	Mean Rep	SD Rep	Replication CV%
A1	18.06	A3	18.11	A5	18.1	18.09	0.03	0.15
B1	18.1	B3	17.8	B5	17.83	17.91	0.17	0.92
C1	17.93	C3	18.01	C5	17.98	17.97	0.04	0.22
D1	18	D3	17.92	D5	17.95	17.96	0.04	0.23
E1	18.01	E3	18.15	E5	18.03	18.06	0.08	0.42
F1	18.03	F3	17.83	F5	17.9	17.92	0.10	0.57
G1	18.08	G3	17.94	G5	18.14	18.05	0.10	0.57
H1	18.05	H3	18.07	H5	17.94	18.02	0.07	0.39
Mean	18.0325		17.9788		17.9838			
SD	0.05339		0.12811		0.10267			
Inter-channel CV%	0.29605		0.71257		0.5709			
A2	24.57	A4	24.39	A6	24.37	24.44	0.11	0.45
B2	24.58	B4	24.47	B6	24.46	24.50	0.07	0.27
C2	24.73	C4	24.54	C6	24.39	24.55	0.17	0.69
D2	24.56	D4	24.45	D6	24.53	24.51	0.06	0.23
E2	24.52	E4	24.53	E6	24.45	24.50	0.04	0.18
F2	24.36	F4	24.38	F6	24.35	24.36	0.02	0.06
G2	24.54	G4	24.58	G6	24.4	24.51	0.09	0.39
H2	24.55	H4	24.51	H6	24.54	24.53	0.02	0.08
Mean	24.5513		24.4813		24.4363			
SD	0.10063		0.0718		0.0713			
Inter-channel CV%	0.40989		0.29329		0.29179			

Replicates obtained from each channel in the amplification of the S10 gene (2 µL of cDNA) provide CV% values ranging from 0.15 to 0.57 indicating very precise liquid handling. Similarly, the CV% among the 8 channels ranged from 0.29 to 0.71 suggesting a high degree of accuracy and reproducibility of all 8 channels of the workstation. Similarly, low CV% were obtained in the amplification of the SF1a gene.

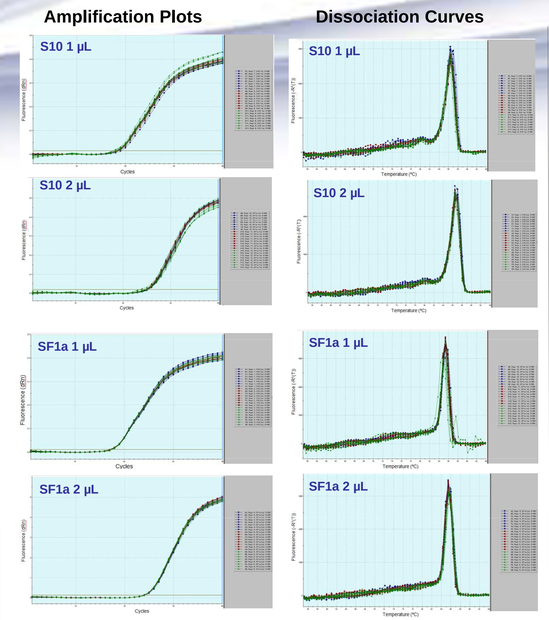

Figure 4: Amplification plots and dissociation curves from the amplification of the S10 and SF1a genes (1 µL or 2 µL of cDNA templates). The data from the replicates and inter-channel analysis indicate that all channels dispensed accurate and precise volumes. Furthermore, same-size amplicons were generated along the deck conditions. These data also suggest no cross-contamination in the automated setup.

VI. Conclusion

Validation of the VERSA 10 Workstation was successfully completed for the real-time PCR setup of cDNA from two trout genes. CV% values of less than 4% were consistently achieved using 1 µL and 2 µL volumes.

VII. Acknowledgements

The authors acknowledge Richard Chea for his valuable contribution.

VIII. References

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