Automated Reaction Set up for Whole-Genome Amplification and Validation with β-actin Gene Using VERSA Mini PCR Set up Workstation

Sikander Gill, Rajwant Gill, Joy Goswami and Dong Liang

Aurora Biomed Inc, Vancouver, BC, Canada

I. Abstract

Downstream genetic analysis is emerging as a tool for understanding genomics. Normally, such large-scale studies identifying genes for particular purposes are hampered by the limited amount of source DNA and throughput. In such processes, task repetitiveness and sample numbers above a certain limit also make the performance difficult and time-consuming. Therefore, in the present studies, automation of the DNA amplification and WGA with VERSA Mini PCR Set up Workstation was carried to produce a large supply of DNA. The results indicated that the automation was successful for the amplification of DNA from buccal cells. Verification of the amplification and representation in the WGA library was carried by amplifying B-actin. The automated results were comparable with manual performance.

II. Introduction

Automation of polymerase chain reaction (PCR) set up has become an important tool for modern genomics to produce high-throughput quality data at a reduced cost[1]. Manual PCR reaction set up is tedious and time consuming as it requires the mixture of multiple reagents in specific ratios to achieve the proper master mix (MM) for amplification. Moreover, the exact volumes of the reagents and buffers depend upon the total number of samples to be amplified and the desired final concentration of sample DNA. Genomic analysis is also hampered by the number of samples to be examined and the availability of sufficient quantities of genomic DNA. Thus automation of such procedures eliminates both human error and contamination problems generally associated with manual set up. To provide solution to such protocols, Aurora Biomed's VERSA Mini PCR Set up Workstation was developed for the preparation of the reagents and samples for amplification protocols directly in a thermocycler 96/384-well plate. To validate this workstation, set up of human B-actin gene and human whole genome amplification[2] were carried out.

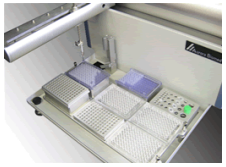
III. Objectives

- To determine the reproducibility of VERSA-prepared amplification
- To check for cross contaminations among DNA samples
- To confirm the stability of the MM when MM is distributed in the entire 96-well reaction plate prior to the addition of DNA samples
- To determine the duration of reaction set up for one 96-well PCR plate
- To validate automation of the set up for whole genome amplification
- To determine the accuracy of delivery of the PCR reagents

IV. Materials & Methods

The validation of VERSA Mini PCR Set up Workstation was carried by using REDExtract-N-Amp Blood PCR kit (Sigma-Aldrich Corp., St Louis, MO, USA) and PCR Core System-II kit (Promega Corp., Madison, WI, USA).

VERSA Mini PCR Set up Workstation



A. B-actin gene amplification: Human genomic DNA (gDNA) isolated from buccal cells was used as a sample for amplification. A 234 bp amplicon of the B-actin gene, a housekeeping gene, was amplified using specific primers. DNA was quantified using spectrophotometer. Controls were run with manual pipettor.

1. **Preparation of Master mix:** The MM was prepared with VERSA aspirating appropriate reagents (Table 1) from the vials placed in the designated slots of cooling block at 4° C.
2. **Multi-dispensing MM:** Following automated mixing, the MM was multi-dispensed to the desired wells of reaction plate (20uL reaction) on deck # 5.
3. **DNA samples:** The desired volume of gDNA sample provided in a 96-well plate was added to the corresponding wells, or from random wells to continuous row or column of 96/384-well reaction plate on deck # 5.
4. **Mixing:** Reaction samples were mixed by pipetting.
5. **Amplification:** Amplification was carried using a MyCycler or iCycler (BioRad Laboratories, ON, Canada) for 96- and 384-well formats, respectively (Table 2).
6. **Cross contamination check:** Cross contamination of the DNA samples was checked by aspirating DNA samples alternating with negative controls.
7. **Stability of MM over time:** MM was distributed to the entire 96-well reaction plate, prior to the addition of DNA samples.
8. **Electrophoresis:** Electrophoresis was carried in agarose (1%) gel. A 50 bp DNA ladder was used.

B. Whole genome amplification: GenomePlex® WGA-I kit (Sigma-Aldrich, St. Louis, MO, USA) was used as follows:

1. **Manually performed steps:** Human gDNA sample isolated from buccal cells was subjected to random fragmentation. The 1X library of small fragments was converted to PCR-amplifiable OmniPlex library molecules by flanking with universal priming sites.
2. **VERSA Mini PCR Set up Workstation steps**: 96-well reaction plate (25 µL reactions) was set up with VERSA according to Table 1. DNA sample (10 µL, 10ng) from the 1X OmniPlex Library molecules was used for amplification.
3. **Amplification:** The reaction plate was amplified in MyCycler for a limited number of cycles (Table 2).
4. **Verification:** To check the representation of B-actin gene in the amplified library, VERSA was used to set up the reaction plate using the amplified library as the DNA source for 234 bp amplicons from B-actin gene.
5. **Electrophoresis:** Electrophoresis was carried in agarose (1.5%) gel. A 100-3000 bp DNA ladder was used.

Table 1: Reagent volumes

Reagent	REDExtract-N-Amp Blood PCR (µL)	PCR Core System II (µL)	WGA (µL)
10X Buffer	-	2.0	-
dNTPs	-	0.5	-
Taq polymerase	-	0.25	1.5
Nuclease free water	6	13.25	16
Primers: FP+RP FP RP	1 1	2	-
ReadyMix	10	-	2.5
DNA template	2	2	5
Total volume	20	20	25

Table 2: Thermocycler conditions

Step	REDExtract-N-Amp or PCR Core System II			Whole Genome Amplification		
	Temp (° C)	Time (min)	Cycles	Temp (° C)	Time (min)	Cycles
Initial denaturation	95	2	1	95	3	1
Denaturation	95	1	40	94	1	14
Annealing	55	1		65	5	
Extension	72	1		65	5	
Final Extension	72	5	1	72	5	1
Storage (short duration)	4	Hold		4	Hold	

V. Results

A. Did the VERSA Mini PCR Set up Workstation generate reproducible results in 96-well format?

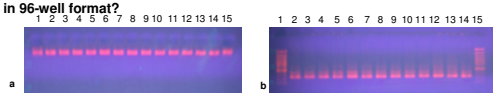


Figure 1. (a) REDExtract-N-Amp Blood PCR kit: 20µL reaction (10 min electrophoresis)- Lane 1 & 15: DNA Ladder, lane 2-12: automated reaction set up, and 13-14: manual reaction set up. (b) PCR Core System-II 20µL (30 min electrophoresis)- Lane 1 & 15: DNA Ladder, lane 2-12: automated reaction set up, and 13-14: manual reaction set up. The amplification was carried using MyCycler (96 well). The accuracy and precision of volume delivery of the reagents is reflected by the quality, uniform amplification and qualitative appearance of amplicon (234 bp) bands from human B-actin for both the kits.

B. Did the VERSA Mini PCR Set up Workstation generate reproducible results in 384 well format?

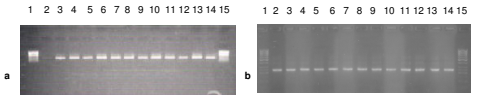


Figure 2. (a) REDExtract-N-Amp Blood PCR kit: 20µL reaction- Lane 1 & 15: DNA Ladder, lane 2-12: automated reaction set up, and 13-14: manual reaction set up. (b) PCR Core System-II: 20ul reaction- Lane 1 & 15: DNA Ladder, lane 2-12: automated reaction set up, and 13-14: manual reaction set up. The amplification was carried with iCycler (384 well). The uniform amplification and qualitative appearance of amplicon (234 bp) bands from human B-actin presented for both the kits shows the quality of reagent handling. It also indicates the accuracy and precision of volume delivery of the reagents.

C. Was any cross-contamination of DNA samples detected when using VERSA for reaction set up?

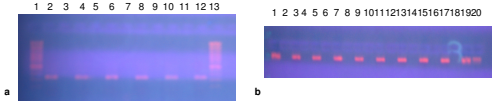


Figure 3. Cross-contamination analysis carried out by alternating wells of positive control (DNA sample) or negative control (blank reagent) using automated operation. (a) REDExtract-N-Amp Blood DNA kit: 20 µL reaction- Lane 1 & 13: DNA Ladder, lane 2-12: automated reaction set up. (b) PCR Core System-II kit: 20 µL- reaction- Lane 1 & 20: DNA Ladder, lane 2-12: automated reaction set up. The amplification was carried using MyCycler (96 well). The presence and absence of amplicon (234 bp) bands in the respective wells suggest no cross-contamination of DNA samples during the automation of the liquid handling by VERSA.

D. How stable was the MM upon its distribution in the entire 96-well plate prior to the addition of DNA samples?

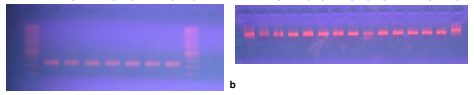


Figure 4. (a) REDExtract-N-Amp Blood DNA kit: Lane 1 & 9: DNA Ladder, lane 2-8: automated reaction set up from well # A1, A12; E1, E6, E12, H1, and H12. (b) PCR Core System-II kit: Lane 1 & 15: DNA Ladder, lane 2-13: automated reaction set up from well # A1, A6, A12, D1, D6, D12, F1, F6, F12, H1, H6, and H12. The amplification was carried using MyCycler (96-well). These results indicates that the MM remains stable and active for the entire duration of the reaction set up. The reaction set up for 96-well format takes about 35 minutes.

E. Can VERSA generate a representative library from whole genome amplification of human gDNA?

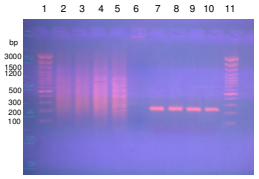


Figure 5. Whole Genome Amplification (WGA-I kit): Lane 1 and 11: DNA ladder, lane 2-3: automated WGA from control DNA supplied by the vendor, lane 4-5: automated WGA from experimental DNA sample isolated with Promega's gDNA kit, lane 6: experimental DNA sample, lane 7-8: amplicon of B-actin from automated reaction set up from WGA product of lane 4 and 5 respectively, and lane 9-10: B-actin amplicon from manually prepared reaction. The data shows that the workstation can perform such applications and the amplified library was confirmed to represent B-actin gene. Thus VERSA can prove as a high-throughput system for the rapid and highly representative, amplification of genomic DNA from trace amounts of starting materials.

VI. Conclusion

1. The data shows that the automation of PCR set up and whole genome amplification can be reliably carried with VERSA Mini PCR Set up Workstation.

2. Precision and accuracy of the aspiration and delivery capabilities of the reagents with the workstation indicate that similar protocols of molecular biology can be performed with this system.

VII. Acknowledgements

We are also thankful to Alicia Davis and Victor Navasero for technical help.

VIII. References

1. Raisi et al.: J Sep Sci, 2004; 27(4):275-283.
2. Hawkins et al.: Curr Opin Biotechnol, 2002; 13(1):65-67.

*Correspondence should be addressed to Aurora Biomed Inc., 1001 E. Pender St., Vancouver, BC, Canada, V6A 1W2, Tel: 1-604-215-8700, Fax: 1-604-215-9700, info@aurorabiomed.com, www.aurorabiomed.com