July, 2016

Amplicon-based Library Construction for Next Generation Sequencing Using the EzMate™ Automated Pipetting System

-- Application Note from Seeing Bioscience Co., Ltd.

Introduction

Seeing Bioscience (http://www.seeingbioscience.com) is a company that develops the technology for diagnosis of disease using molecular biology and provides comprehensive molecular biology services including next generation sequencing (NGS). In the NGS preparation, amplicon-based library construction is popular to analyze trace and targeted gene sequence. It is common to prepare hundreds of amplicon libraries for an amplicon-based NGS service case.

In this application note, we demonstrate that the EzMate™ Automated Pipetting System is an ideal tool for the amplicon-based NGS preparation. It does well in amplicon library construction and provides a cool environment for *Taq* polymerase.

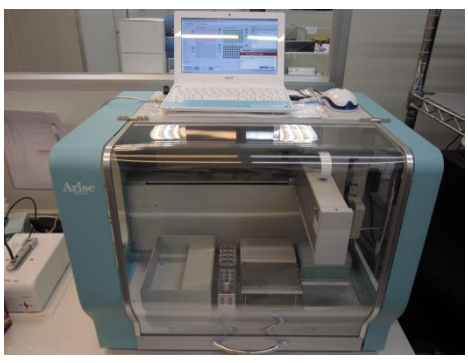
Materials

Reagents and Consumables:

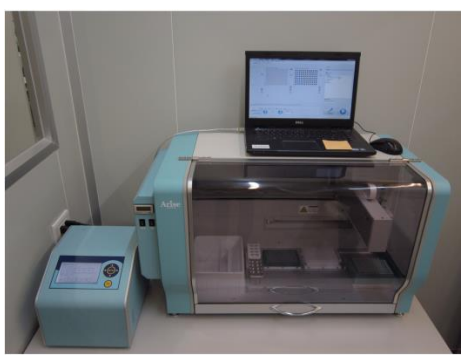
- FFPE DNA samples
- Nested PCR primers (outer)
- Barcode PCR primers (inner)
- Recipe of PCR master mix: Betaine, dNTP, 10X Supertherm GOLD buffer, Supertherm GOLD *Taq* polymerase
- 96-well and 384-well PCR plates

Equipment:

- Arise EzMate™ 401 & 601s Automated Pipetting System
- 8-channel, 50µl Automatic Pipetting Module (APM50-8)
- Arise CoolBlock™ 384 adapter for 384-well PCR plate
- General laboratory equipments



EzMate™ 401



EzMate™ 601s

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Methods

1. Use a manual pipette to mix PCR master mix and outer PCR primers in a 2ml screw cap tube. Transfer the mixture to one column of a 96-well PCR microplate.
2. Repeat step 1 to transfer 4 different kinds of PCR mixture, each with one pair of outer PCR primers to a 96-well PCR microplate.
3. Place the 96-well PCR microplate with mixture on area C of the EzMate™ 601s. Transfer the 9μl PCR mixture with outer primers to a 384-well PCR microplate on area A (Fig. 1).

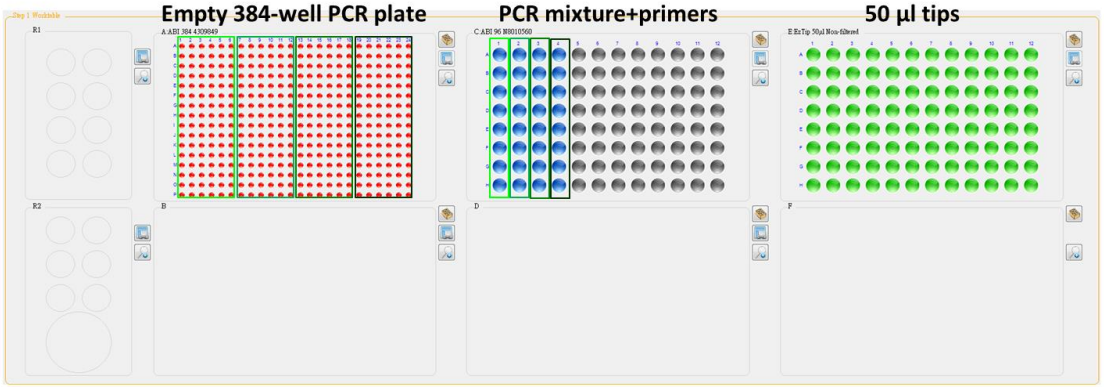

Fig. 1 Layout of the EzMate™ 601s.

4. Place the 384-well PCR microplate with PCR mixture on area A and the 96-well PCR microplates with 96 DNA samples on area C of the EzMate™ 601s. Transfer the 1μl DNA samples to a 384-well PCR microplate on area A (Fig. 2).

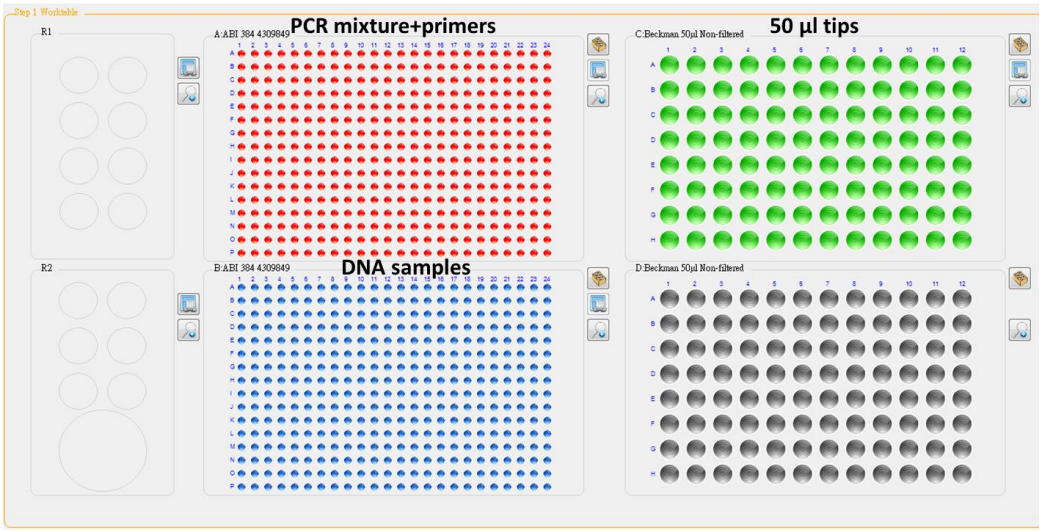

Fig. 2 Layout of the EzMate™ 401.

5. After the PCR assay is completed, place the 384-well PCR microplate with 384 PCR products (which will be used as DNA samples to perform the PCR assay with inner PCR primers) on area C of the EzMate™ 401.
6. Repeat steps 1~3, but add inner primers to prepare the PCR master mix with inner PCR primers for the second PCR.
7. Transfer the 1μl PCR products in the 384-well PCR microplates on area C to the 384-well PCR microplate on area A of the EzMate™ 401. Then, perform the PCR assay.
8. Repeat above steps for all the other samples and primer pairs.

Result and Discussion

The data in Fig. 3 indicates that the EzMate™ series can handle fatiguing pipetting tasks in amplicon library construction and performs with high accuracy and good precision.

By introducing the EzMate™ series to do the amplicon-based library construction, one can obtain a high-quality sequencing library which is very important for NGS, and prevent manual pipetting errors so as to save time and money. In this experiment, the EzMate™ 601s with Active Cooling and Heating Module (ACHM) and the Arise CoolBlock™ adapter keeps the polymerase at an appropriate cool temperature that is important for PCR amplification and sequencing.

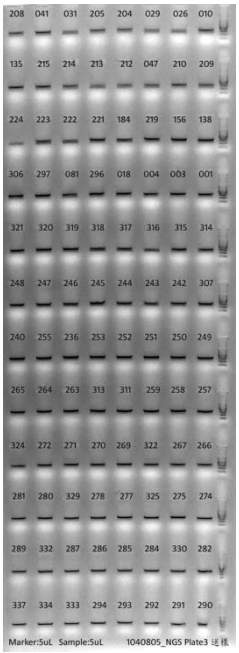

Fig. 3 Analysis of PCR products using agarose gel.