# Automated PCR setup using the Hydra II eDrop®

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## Abstract

Hydra II eDrop is a bench top instrument capable of performing 96 or 384 liquid handling operations at sub-micro liter levels. Equipped with 96/384 and single channel micro dispensers plus a user friendly ControlMate™ software, the Hydra II eDrop has the capacity to perform several biological applications. In this report, we discuss a generic process to setup a PCR using the Hydra II eDrop. The setup is validated by subjecting the reaction to amplify β-actin gene using human genomic DNA as a template. The process is reliable and significantly reduced manual intervention.

## Introduction

Polymerase chain reaction (PCR) is a routinely used technique in biological laboratories for diverse applications. A typical PCR setup involves addition of nucleotide triphosphates and buffers along with a pair of complementary stretches of oligonucleotides to a DNA template. The mixture is then subjected to a series of heating and cooling cycles to amplify specific regions of the template DNA by several thousand folds. The operational process of PCR can broadly be divided into two stages. Stage 1, which is the PCR setup usually performed manually with pipettes and Stage 2, which is performed on a thermocycler. While the process in stage 2 requires negligible manual intervention, the process in stage 1 involves mixing of different ingredients in appropriate volumes. Based on the throughput in a specific laboratory, repeated pipetting of ingredients multiple times can be cumbersome and error prone. Moreover, use of multichannel pipettes may not be appropriate due to loss of expensive reagents in the reagent reservoir. For the purpose of minimizing the tedium involved in manual PCR setup, we attempted to utilize the capabilities of Hydra II eDrop to automate the setup of the reaction. The capacity of Hydra II eDrop to dispense sub-microliter and microliter volumes of reagents makes it an appropriate instrument for setting up analytical as well as preparative scale PCRs.

Briefly, Hydra II eDrop is a compact bench top instrument designed to perform liquid handling operations in nano and micro liter volumes (see specification sheet for details). The Hydra II eDrop is equipped with 96-channel syringe array and single channel non-contact micro dispensers both of which have been engineered and validated to accurately aspirate/dispense biological samples of different physicochemical properties. The combined usage of these dispensers enables users to perform both single and multi well applications simultaneously. The Hydra II eDrop is programmed using ControlMate software. ControlMate is designed for ease of use and offers several options to optimize the process to the minute level and achieve highly reliable results. *Figure 1* shows the various components of Hydra II eDrop and the programming interface of ControlMate respectively.

Hydra II eDrop exhibits several features that can readily be adapted to various applications. In the current study, we demonstrated and validated the use of Hydra II eDrop for setting up a typical PCR. In this setup, we observed a throughput time of less than twenty seconds per well.

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## Materials used in the study

### Chemicals and Reagents

- Degassed distilled water
- 80% ethyl alcohol
- dNTPs (Fermentas)
- MgCl2
- Taq DNA polymerase (Fermentas)
- Primers for β-actin (Biosource)
- Human genomic DNA (Novagen)

### Instruments /material

- Hydra II eDrop (Matrix Technologies)
- 96-well PCR plate and seal
- Thermocycler (PerkinElmer)

## Results

The Hydra II eDrop was programmed to automate the PCR setup. The Hydra II eDrop was primed with degassed distilled water using the procedure described in the manual. A generic program for the automated process was developed using ControlMate software. Movement and speed of the micro dispenser were adjusted using the select and drop features of the software. The stage positions were appropriately assigned using the programming interface. Multiple dispenses were achieved by grouping the commands and looping the program cycles appropriately based on the number of wells in use. The ControlMate software allows users to select specific wells in a plate. Message commands were displayed to enable the user to understand every module of the process. Briefly, the program can be discussed in two broad stages, one involving dispensing genomic DNA and the other involving addition of reagent mix. The Hydra II eDrop single channel nozzle was washed three times with 120µl of distilled water between dispenses of different concentrations to prevent cross contamination. Pause commands were included in the program to stop the process for intermittent changing of reagents whenever necessary. While walk-away automation of the process could be achieved using different tube positions, the process described here was designed using a single tube position for all aspirations with pause commands at the respective steps. This design enabled users to interact with the instrument periodically and understand every movement on Hydra II eDrop. All the programming features are very flexible and can be modified according to the specific process. The user is advised to refer to the manual for more details on instrumentation and programming.

As a proof of concept experiment, partial beta actin gene from human genomic DNA was amplified using synthetic oligonucleotides. Three different concentrations of genomic DNA were used as templates in the reaction. The genomic DNA and ingredients for the PCR were added using the single channel dispenser of Hydra II eDrop. The process started with addition of 1µl of genomic DNA of different dilutions into three independent wells of a 96-well plate. A total reaction volume of 20µl consisting of 1µl of genomic DNA and 19µl of master mix was used. The nozzle was subjected to three wash cycles with 120µl of distilled water each time to prevent cross contamination between additions of template DNA samples. The PCR was performed using a thermocycler and a program used to amplify the β-actin gene. The PCR included thirty cycles with melting temperature of 94°C for 45 seconds, annealing temperature of 55°C for 45 seconds and a synthesis temperature of 72°C for 45 seconds. An initial melting temperature of 94°C and a final extension step of 5 minutes at 72°C were also included in the program. The PCRs were subjected to agarose gel analysis and are shown in *Figure 2*.

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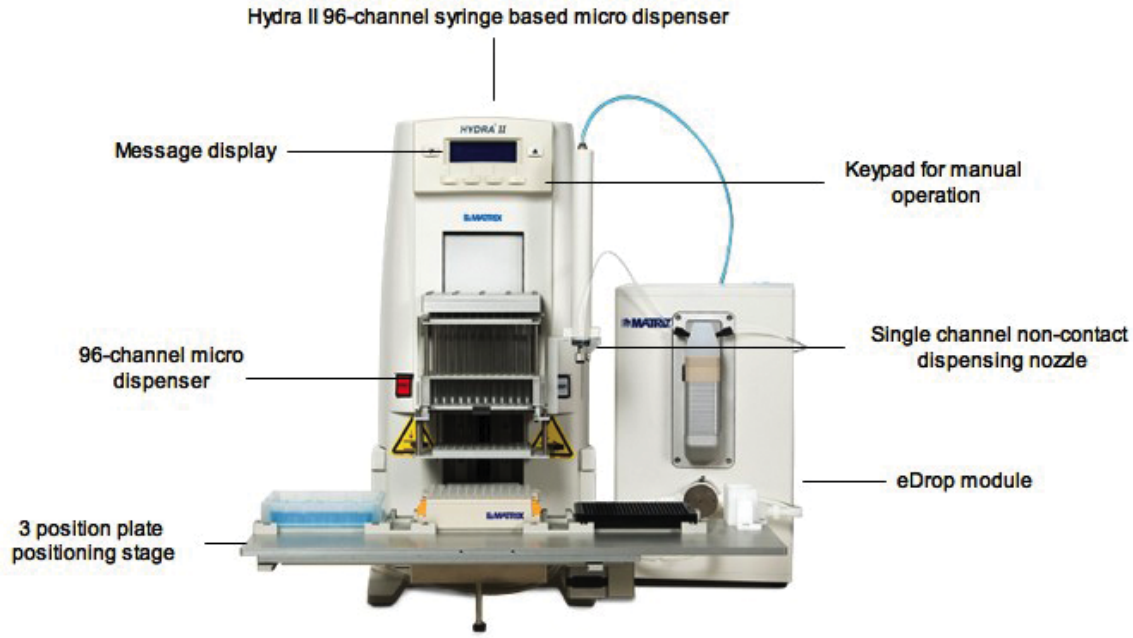


**Fig 1: Hydra II eDrop**

## Conclusion

A generic automated process for a typical PCR setup on Hydra II eDrop has been developed and validated. The results indicate that Hydra II eDrop can be an efficient tool to automate the setup of PCRs. The operations on Hydra II eDrop are more accurate and minimize the tedium involved in manual operations. The operational procedure and program used in this study can be customized to a wide range of PCR and other biological applications.

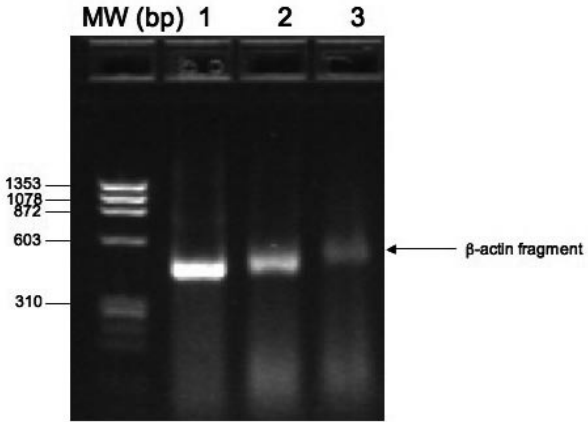


**Fig 2: DNA agarose gel showing amplification of b-actin from human genomic DNA. Samples 1, 2 and 3 indicate 246, 2.46 and 0.246 ug/ml of the template genomic DNA used in the reaction. The entire PCR setup was automated using the Hydra II eDrop and the PCR reaction was performed on a thermocycler.**

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