Using the SOLO for PCR Preparation

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Introduction

The polymerase chain reaction (PCR) is one of the most widely used techniques in the field of molecular biology. PCR makes it possible to produce useable quantities of any particular sequence of DNA by systematic amplification of a single sample. The basic process involves mixing the template sequence with a collection of the four deoxyribose-nucleotides and DNA polymerase along with a starting primer sequence, salts and buffer. The sample is put in a thermocycler and the temperature is varied through a cycle of three steps:

1. Above 95°C to denature the double-helix
2. 50-65°C to allow the primer to anneal to the single strands.
3. 70-75°C where the DNA polymerase elongates the complementary chain to form a second copy of the original sample.

The three steps are repeated to produce four copies of the sequence, then eight copies, and so on. A typical run consists of 20 to 40 cycles, after which the sample is cooled to 4-15°C until it is ready for purification.

Automation of the PCR Procedure

Automation is required when a large number of DNA sequences need to be amplified. These reactions are each run on a scale that is consistent with microplate technology, which means 96 or 384 samples can be analyzed in a single plate.

The typical microplate-based PCR protocols involve three basic steps:

1. Prepare the aqueous mixture of nucleotides, polymerase, primers, and sample.
2. Systematic heating and cooling in the thermocycler to create complementary DNA sequences and denature the corresponding double helix.
3. Purification of the amplified DNA samples so they can be used in downstream applications, such as sequence determination or protein expression.

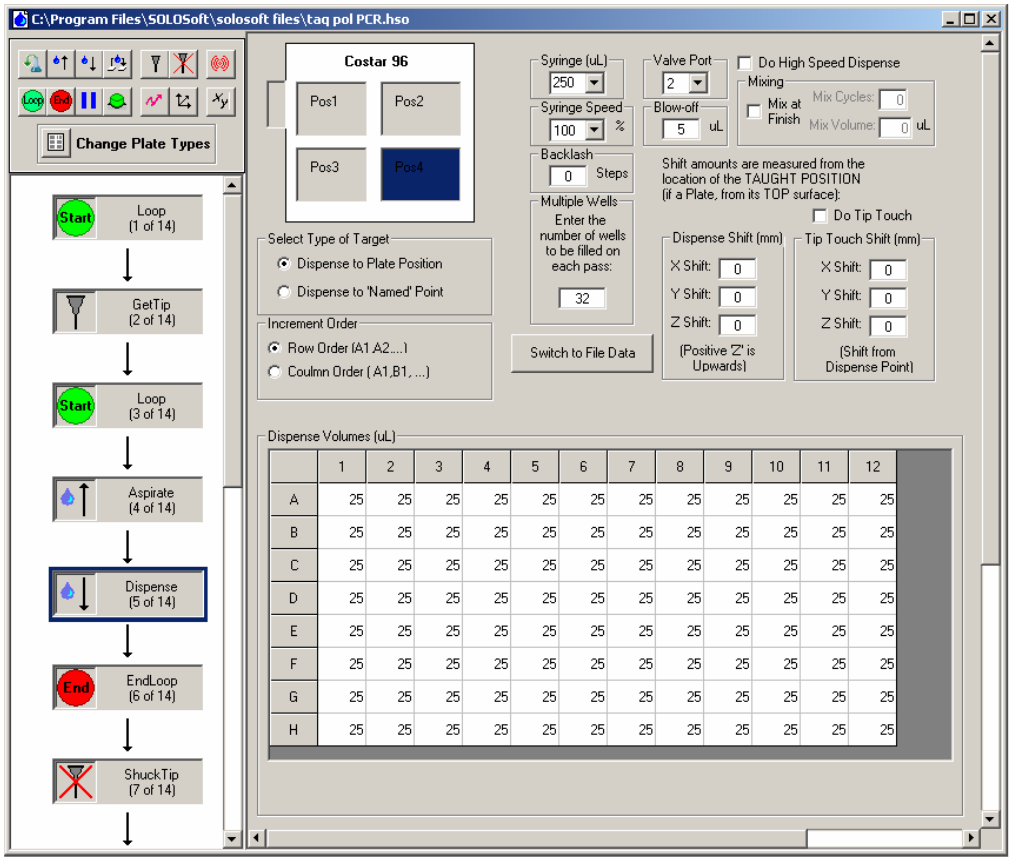
In this document, we focus on how Hudson's SOLO robotic pipettor can used to prepare microplates for the PCR reaction.

Using the SOLO for PCR Preparation

Hudson's SOLO pipettor is well suited for the automatic preparation of samples for PCR reactions, whether as a stand-alone unit, or as part of a larger application – such as DNA sequence analysis. The process described here starts with quantities of sample DNA sequences that have been prepared/isolated and purified, usually via spin column or magnetic bead derived protocols. The result of the process will be unsealed, 96-well plates, containing all of the PCR components in the appropriate concentrations, ready for manual transfer to a thermocycler.

The sample protocol described here is a common method using Taq DNA Polymerase, and is based on 96 well plate samples with final volumes of 50 µL/well. In this approach, 25mM Magnesium Chloride is included in the master mix. The PCR procedure is notoriously dependent on the concentration of this particular component, and some labs routinely carry out multiple runs per sample with systematic variations in the concentration of the salt.

The entire protocol is easily setup in SOLOSoft, the scheduler software that comes with the SOLO. The program's user interface allows the user to drag and drop the various pipettor functions into a flow chart. Each function includes a corresponding dialog window that allows the user to define exactly how the SOLO carries out that step. The following figure shows the features available in the dispense dialog window:



Protocol Setup

The first step is to prepare a master mix solution. A typical run might include 25 µL of master mix for each well, or 2.4 µL per 96-well plate. One nest of the SOLO will be dedicated to the supply of fixed reagents. The total number of plates in a run will determine the nature of the appropriate source plate. For example, if a single stack of 30 plates were being studied, 72 µL of master mix would be required, which a single-well assay dish could accommodate (eg. NUNC 267060).

Reagent	Final Concentration
Sterile deionized water	
10X *Taq* buffer	1X
2 mM dNTP mix	0.2 mM of each
Primer I	0.1-1 µM
Primer II	0.1-1 µM
Taq DNA Polymerase	1.25 u / 50 µL
25 mM $MgCl_2$	1-4 mM

The DNA samples are provided in individual wells of a 96-well plate, and are usually in solution in elution buffer. A typical run will contain nanogram to low microgram quantities of template DNA. These samples can be further diluted with sterile deionized water, or the water can be added in advance. In this protocol, the samples are pre-diluted to 25 µL.

SOLO Nest Setup

The standard SOLO includes 4 nest positions. In the PCR application, the master mix is placed in the first nest. A wide variety of receptacles can be used to store this reagent, including a single-well assay dish (eg. NUNC 267060), or any variety of multi-well plates, depending on the total volume needed in the experiment. Alternatively, there are a number of SBS-format containers that can hold small jars, vials, or Eppendorf tubes. In a second nest position are DNA samples stored in a 96-well plate. The third nest position contains disposable pipette tips, and the final nest contains the 96-well plates to be used for the PCR reaction. This nest is typically equipped with the SOLO's shaker nest (P/N 800330) to provide the required level of mixing before submission to the thermocycler. The SOLO setup is depicted below:

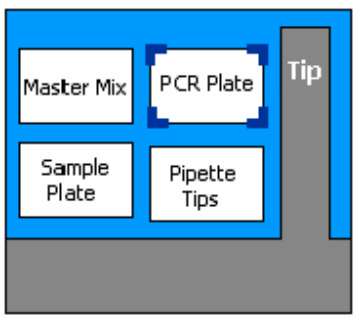
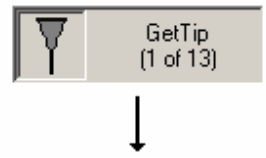
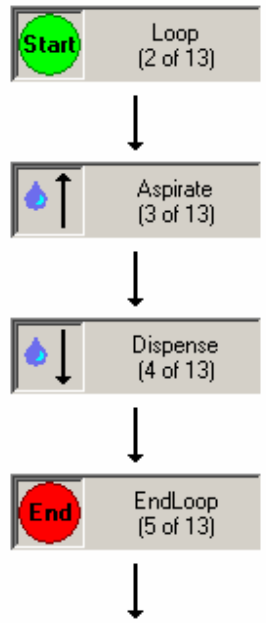
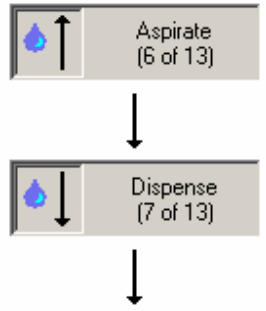
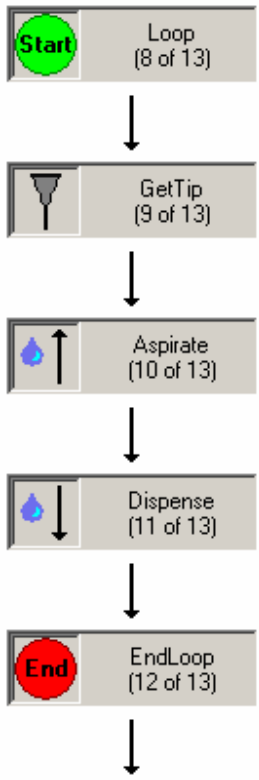
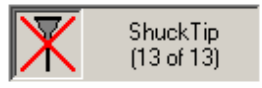


Plate Definitions on the SOLO setup for PCR Preparation

SOLOSoft Protocol

The following protocol shows how SOLOSoft would direct the SOLO to prepare a plate for a PCR run. The method is broken down into several components, which are described briefly below:

	In the first step, the SOLO gets a new tip, and is instructed where to get it from. The SOLO will automatically shuck any previously attached tip.
	A new loop begins which controls the transfer of the master mix to each of the wells of the reaction plate. This SOLO is equipped with a 1 milliliter syringe, but 2.4 milliliters are required to fill all the wells in the plate. So the procedure is divided into 3 equal passes, in which 800 microliters are aspirated (step 4), and 25 microliters are dispensed into each of 32 wells (step 5). The aspirate step offers several options, including syringe speed, pre-aspiration, aspiration height, and the ability to mix the reagent several times before beginning. The dispense step also contains many options that can be used to fine-tune the accuracy and speed of the dispense.
	In this step, we aspirate 25 microliters of DNA sample from the first well in nest 2 and transfer it to the first well in the reaction plate in nest 4.

	We need to repeat the above transfer 95 more times, but unlike the first reagent transfer, avoiding contamination is crucial, so this loop adds the replacement of the pipette tip before each transfer. As above, the GetTip step automatically discards any tip that is on the tip mandrel, then secures another tip from the tipbox.
	This step completes the preparation of a single PCR reaction plate, and discards the last tip used on the reaction plate so it does not remain on the mandrel.

The PCR reaction plate can now be removed from the SOLO and is ready, after sealing, for the thermocycler.