Using the SOLO for PCR Automation

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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.

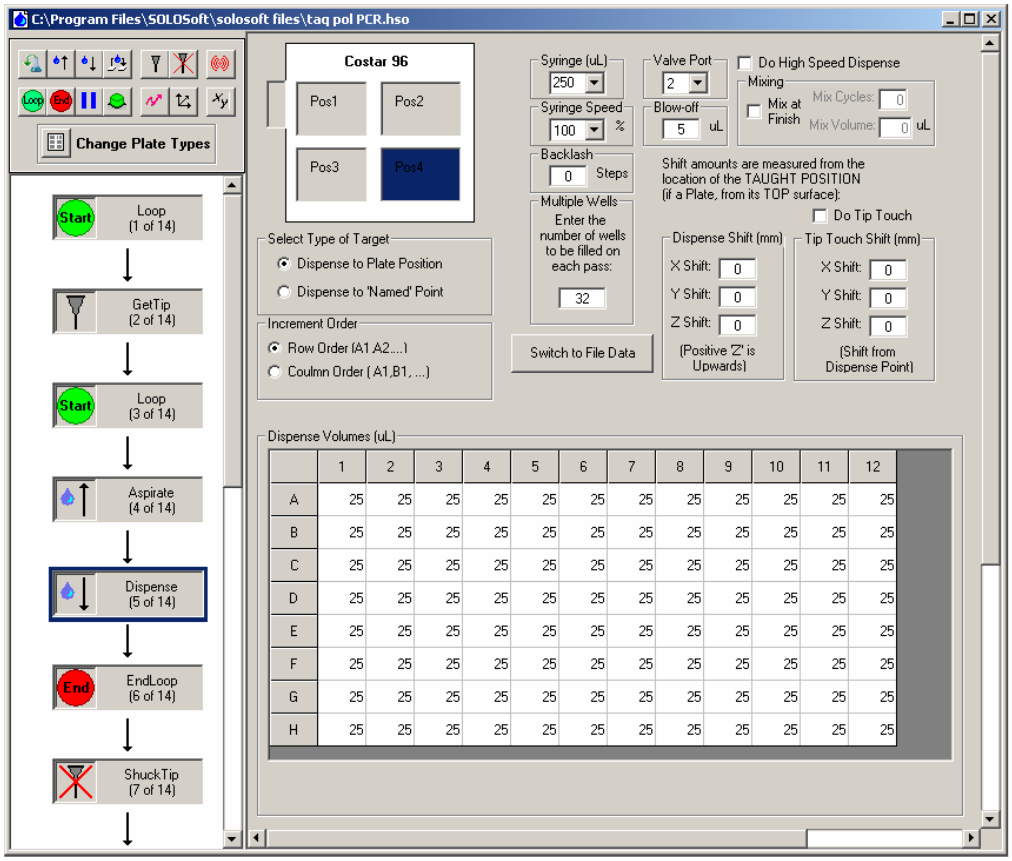
In this document, we focus on the ways Hudson's SOLO robotic pipettor can prepare microplates for PCR reactions and then purify the results for downstream processing (steps 1 and 3 above).

Step One: 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 24 mL 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:

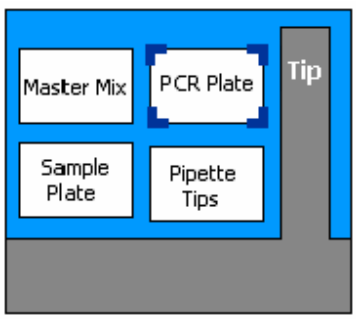
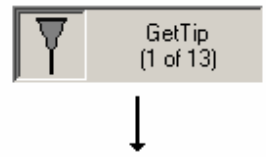
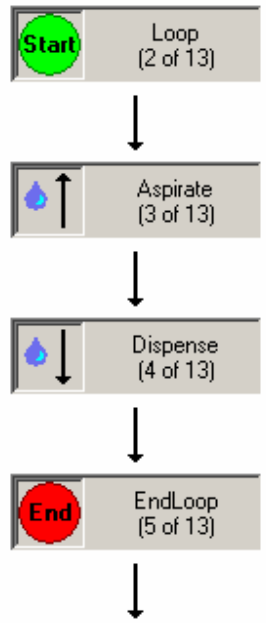
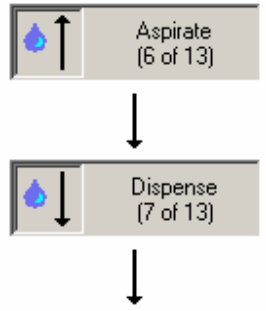
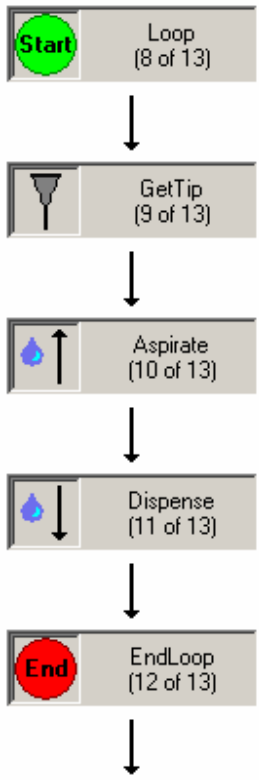
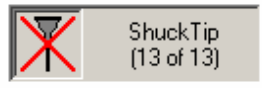


Plate Definitions on the SOLO setup for PCR Preparation

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.

Step Two: Running the PCR Reaction

In many cases, it is feasible to manually remove the plate from the deck of the SOLO, add a plate seal, and physically transfer it to the thermocycler for processing. If sample size is large enough to justify the expense of further automation, it is possible to add a robotic arm, such as Hudson's PlateCrane EX, and an automatic plate sealer. The PlateCrane can move the plate to the sealer, and then move it to the thermocycler. The SoftLinx software, described in the purification protocol below, can manage this process as well.

Step Three: Using the SOLO for PCR Purification

The SOLO described above is sufficiently equipped to handle the preparation of samples for PCR; however, following amplification in the thermocycler, additional options are required to purify the reaction mixtures to a level suitable for most downstream applications, such as genomic sequencing or bacterial transformation. Most laboratories use some form of separation procedure to remove unreacted material, reagents and side products from the desired samples. One of the most common approaches uses pre-designed kits such as the QIAquick MinElute PCR Purification kit supplied by Qiagen. The sample protocol described here is based on this kit, and uses a PCR reaction volume of 50 microliters per well.

Protocol Setup

The MinElute Purification protocol contains two steps. In the first step, The PCR reaction mixture is passed through a filter plate containing a silica membrane assembly that strongly binds DNA in an aqueous buffer solution with a high-salt concentration. The primers, enzymes, nucleic acid fragments, and the remaining reaction components and resulting impurities all pass through the silica material and are collected in a waste receptacle and discarded. In the second step, the silica membrane assembly is washed with a second, low-salt aqueous buffer that removes the pure DNA.

SOLO Nest Setup

In order to provide full automation of PCR purification, we have added two automated vacuum nests to the left of the SOLO's deck (Hudson's VaryVac, P/N 55001 and P/N 55002). The SOLO axis that moves the pipette tip has been extended to reach these nests. A PlateCrane EX robot arm is included to fully automate the process by transferring plates between the sample and vacuum nests. The PlateCrane is equipped with five microplate storage stacks for storing plates tips and samples for running multiple plates. The first vacuum nest is used to pass the newly formed DNA through filter plates containing the silica membrane assembly. The filtrate from this nest is always discarded, so a non-motorized version of the VaryVac is used. The second nest is used to elute pure DNA, so a motorized VaryVac is used. It opens to allow insertion and removal of collection plates.

Two of the nests on the deck of the SOLO are used to provide the high-salt wash buffer and low-salt eluent buffer. A wide variety of receptacles can be used to store these reagents, such as a single-well assay dish (eg. NUNC 267060), or any variety of multi-well plates, depending on the amount of volume needed in the experiment. The amplified samples obtained from the thermocycler are placed on a third nest, and a box of pipette tips is placed on a last nest.

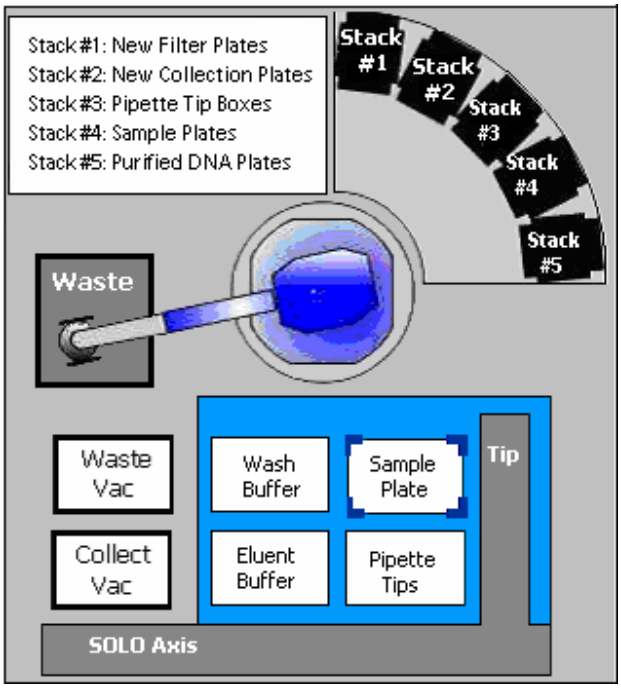
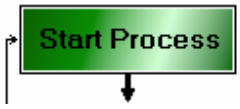
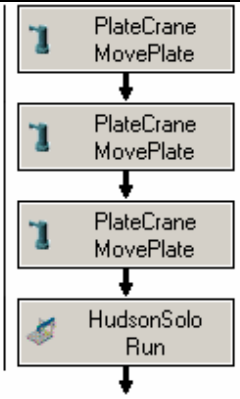
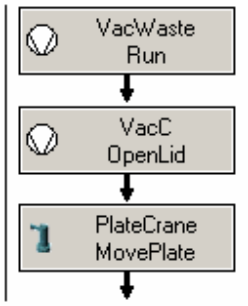
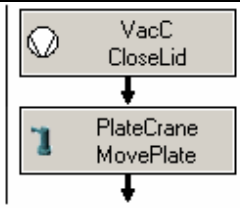
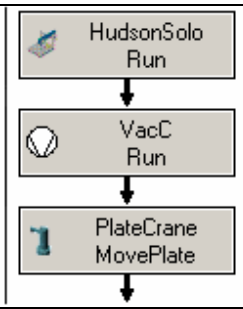
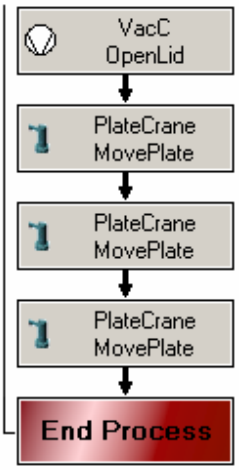


Plate Definitions on the SOLO and PlateCrane EX setup for PCR Purification

SoftLinx Protocol

With the addition of the VaryVac vacuum nests and the PlateCrane, much of the scheduling is handled by Hudson's SoftLinx scheduling software. SoftLinx is capable of integrating hundreds of microplate-based laboratory equipment, and uses a similar drag and drop flow chart approach as described above for SOLOSoft. The following protocol shows how SoftLinx would direct the SOLO and vacuum nests to perform a fully-automated PCR purification. The method is broken down into several components, and described briefly below:

	SoftLinx procedures begin with a "Start Process" method as shown at left. This method is used to define the number of times, or under which conditions, the method should be carried out
	The first "PlateCrane MovePlate" procedure indicates the movement of a filter plate containing the silica membrane assembly from stack #1 to the top of the waste vacuum nest. The second MovePlate procedure indicates the transfer of a sample plate containing the PCR products from stack #4 to the deck of the SOLO. The final MovePlate step retrieves a fresh box of pipette tips from stack #3. We then call a SOLOSoft protocol, which tells the SOLO to add wash buffer to each PCR product sample, and then transfer (aspirate/dispense) the resulting mixture to the filter plate.

	We then run the vacuum, which leaves the original plate containing only the silica membrane assembly and the desired DNA material. The filtrate is discarded during the run. The lid of the second vacuum nest is then opened and the PlateCrane then moves an empty 96-well collection plate from stack #2 to the bottom of the vacuum nest.
	The second vacuum nest is then closed, and the PlateCrane moves the filter plate containing the sample embedded on the silica material from the first to the second vacuum nest.
	The SOLO is now instructed to add the second, low-salt buffer solution. The vacuum on the second nest is then started. When the purified DNA has been vacuumed into the collection plate, the PlateCrane removes the filter plate discards it into the waste container.
	The second vacuum nest is opened and the PlateCrane removes the plate containing the pure DNA material from the lower chamber and placed into stack #5. Next, the PlateCrane removes the empty sample plate from the deck of the SOLO and discards it into the waste container. Finally, the PlateCrane removes the tipbox from the Solo and moves it to waste. This completes the purification procedure.

Additional Automation

The two procedures described above are ideal in situations where a relatively small number of PCR plates are handled in a day. The user places the samples in a plate, puts it on the deck of the SOLO and runs the preparation procedure. The resulting plate is manually taken from the SOLO, sealed, and placed in the thermocycler and the PCR procedure is run. Once completed, the plate is returned to the SOLO and the purification procedure is run.

In laboratories that encounter large quantities of DNA samples, further automation is available to organize, store and distribute large numbers of plates. The entire PCR process can be automated by adding a SoftLinx interface to your thermocycler. In addition to the SOLO, PlateCrane and VaryVac, Hudson Control Group offers microplate bar-coding and sealers, as well as the LabLinx track and storage system, which can greatly expand the level of possible integration. One can further speed up the sample preparation process by adding the twelve-channel MicroFlex dispenser for addition of Master Mix to PCR reaction plates.