

## Using the SOLO for PCR Preparation

Alan H. Katz, PhD

Hudson Control Group

The Hudson SOLO is well suited for the automatic preparation of samples for PCR reactions, whether as a stand-alone unit, or as part of a larger sequence – such as DNA sequence analysis. The stand-alone unit described here, assumes that sufficient quantities of sequences have been prepared/isolated and purified, usually via spin column or magnetic bead derived protocols. At the end of the sequence, unsealed, 96-well plates, containing all of the PCR components in the appropriate concentrations, will be ready for manual transfer to a thermocycler. A number of automation options are mentioned at the end of this document, that are available to further extend the degree of automation of the entire process – including the preparation of the samples, and completion of the protocol

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 50uL/well. In this approach, 25mM MgCl<sub>2</sub> 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 salt concentration. This approach is easily adopted in SOLOSoft, and would involve the use of multiple master mix sources, instead of one.

### Protocol Setup

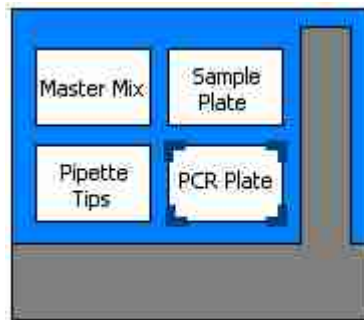
The first step is to prepare a master mix solution. A typical run might include 25uL of master mix for each well, or 1.92mL per 96-well plate. One nest of the SOLO will be dedicated to the supply of fixed reagents. The total number of plates in a run will determine the nature of the appropriate source plate. For example, if a single stack of 30 plates were being studied, 57.6 ml of master mix would be required which a single-well assay dish could accommodate (eg. NUNC 267060).

Reagent	Final concentration
Sterile deionized water	
10X <i>Taq</i> buffer	1X
2 mM dNTP mix	0.2 mM of each
Primer I	0.1-1 µM
Primer II	0.1-1 µM
<i>Taq</i> DNA Polymerase	1.25 u / 50 µl
25 mM MgCl <sub>2</sub>	1-4 mM

The DNA samples are provided in individual wells of a 96-well plate, and are usually dissolved in elution buffer from the previous purification step. 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 separately. In this protocol, the samples are pre-diluted to 25uL.

## SOLO Nest Setup

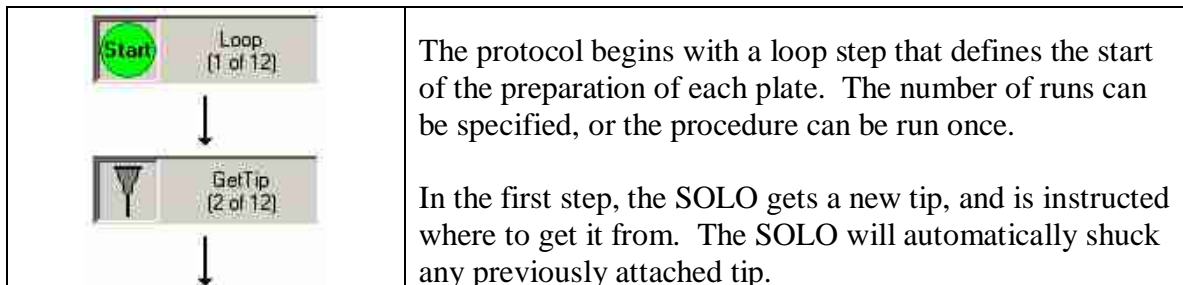
The standard SOLO includes 4 nest positions. In the PCR application, the master mix is placed in the first nest. A wide variety of receptacles can be used to store this reagent, including a single-well assay dish (eg. NUNC 267060), or any variety of multi-well plates, depending on the amount of volume needed in the experiment. Alternatively, there are a number of SBS-format containers that can hold small jars, vials, or Eppendorf tubes. In nest position two are DNA samples stored in 96-well plates. 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 a shaker nest (P/N 800330) to provide the required level of mixing before submission to the thermocycler.



*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:



<pre> graph TD     Start((Start 3 of 12)) --&gt; Aspirate[Aspirate 4 of 12]     Aspirate --&gt; Dispense[Dispense 5 of 12]     Dispense --&gt; EndLoop((EndLoop 6 of 12))     EndLoop --&gt; Start   </pre>	<p>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 the entire plate. So the procedure is divided into 3 equal passes, in which 800 microliters are aspirated (step 4), and 25 microliters is dispensed into 32 wells (step 5).</p> <p>The aspirate step offers several options, including syringe speed, pre-aspiration, aspiration height, and the ability to mix the reagent several times before beginning.</p> <p>The dispense step also contains many options that can be used to fine-tune the accuracy and speed of the process.</p>
<pre> graph TD     ShuckTip[ShuckTip 7 of 12] --&gt; EndLoop7[EndLoop 7 of 12]   </pre>	<p>Once all of the master mix is distributed, the tip is removed in preparation for the next sequence.</p>
<pre> graph TD     Start((Start 8 of 14)) --&gt; GetTip[GetTip 9 of 14]     GetTip --&gt; Aspirate[Aspirate 10 of 14]     Aspirate --&gt; Dispense[Dispense 11 of 14]     Dispense --&gt; ShuckTip[ShuckTip 12 of 14]     ShuckTip --&gt; EndLoop((EndLoop 13 of 14))     EndLoop --&gt; Start   </pre>	<p>In this loop, 25 microliters of DNA sample are aspirated from nest 2 and transferred to the reaction plate in nest 4. Unlike the first reagent transfer, avoiding contamination is crucial, so this loop requires the removal of the pipette tip after each sample transfer.</p>
<pre> graph TD     EndLoop[EndLoop 14 of 14]   </pre>	<p>The final method ends the preparation of the PCR reaction plate.</p>