Helicos™ Low-Volume Sample Loading Protocol

The Low-Volume Sample Loading protocol is an experimental procedure designed for use with the HeliScope™ Sample Loader in situations where sample amounts are very limited, and it is impossible to prepare sufficient diluted sample for the standard (100 µL) loading protocol.

Helicos™ Control Oligonucleotides should always be loaded according to the standard (100 µL) protocol outlined in the HeliScope™ Sample Loader Manual (LB-016). Exemplar and training samples should also be loaded using the 100 µL protocol. Due to the experimental nature of the low-volume loading protocol, it is also strongly recommended that all other samples be loaded using the 100 µL protocol if at all possible.

NOTE: If some samples are being loaded with the standard (100 µL) protocol and some with the low-volume protocol:

- Organize the plate layout such that the samples are split into two blocks, one for the 100 µL samples and one for the 20 µL samples. Marking the transition between these two blocks should help reduce the possibility of double-loading a channel by losing your place.
- Load the 100 µL samples into their respective channels FIRST, according to the standard (100 µL) protocol. This will leave the 100 µL-filled channels at the start of their hybridization when the 20 µL channels are still filled with pre-hybridization buffer. Proper tracking of different sample volumes is critical. Proceed to the steps outlined below for the low-volume (20 µL) samples.
- Start timing the 1 hour hybridization after the last 20 µL sample is loaded. This will lead to varying hybridization times for the samples that have been loaded earlier – it is therefore suggested that the hybridization start time for the first sample be recorded for tracking purposes.

Prepare the Sample (5 - 10 minutes)

Prepare 20 µL of each sample in Hybridization Buffer according to the instructions found in the HeliScope™ Sample Loader Manual (LB-016), Step 1b-2. To prepare the sample for the low-volume loading procedure, dilute the sample in a final volume of at least 10 µL and add an equal volume of Hybridization Buffer.

Hybridize (Hybridization Buffer - 1 hour)

1. Disconnect the vacuum tube from the waste bottle (when looking at the back of the Flowcell Loader it is the line coming out of the right vacuum port) and attach a 1 mL syringe to the end of the tube.

2. Load one channel at a time.

3. Press the button corresponding to the first channel that is to be loaded using 20 µL of sample, and using the syringe, pull all the PreHyb Buffer through the channel until it is empty of liquid.

4. Immediately after pulling the PreHyb Buffer through the channel, dispense 20 µL of sample into the appropriate well of the loading block.

5. Press the button corresponding to the channel position being loaded, and slowly pull the sample into the channel using the syringe. Carefully monitor the progress of the fluid front as it travels through the channel. Pull only enough

sample to just fill the channel. **DO NOT pull the entire sample through the channel.**

IMPORTANT: DO NOT push the sample back into the channel if the sample has traveled too far. Applying positive pressure using the syringe may damage the flow cell and could lead to sample cross-contamination. If the sample has traveled too far, additional sample can be loaded if available; if not, the channel should be left as is, and the channel number recorded. Depending on the extent of the void in the channel, there may be a low density of template molecules in that channel.

NOTE: Careful control of the syringe is necessary during the loading process. Apply only enough vacuum to move the fluid front SLOWLY through the channel.

6. To apply more vacuum to the channel, disconnect the 1mL syringe, then push the plunger back to the zero (0) mL position to reset it. Reattach the syringe.

7. Repeat steps 3 through 6 for the remaining channels that are being loaded according to the low volume (20 µL) protocol.

8. Hybridize at 55° C for 1 hour. Start timing after the last channel is loaded.

9. Reattach the vacuum tube to the waste bottle and proceed to HybWash A as per the standard (100 µL) protocol (found in the HeliScope Sample Loader Manual LB-016).