# Microfluidics with the LabSmith LabPackage

# Making a Microfluidic Injection on a Chip

- Fill a chip
- Place electrodes
- Program the HVS to load and inject a sample
- Visualize your experiment


**LabSmith's LabPackage makes it easy to build, control and monitor simple and complex microfluidic manipulations.**

**This experiment was designed to help you train your laboratory team and test competency on microfluidic techniques. It can also be used to test equipment, chips and reagents to ensure that they are working properly. Though the experiment can be attempted with other equipment, the settings and consumables listed here are specifically for use with LabSmith equipment.**

## Performing a Microfluidic Pinched Injection

### Preparing the Chip

Microfluidic channels on planar substrates (chips) enable the use of electric fields to confine volumes without creating dead volumes or carry over. LabSmith's CapTite™ bonded port connectors and microfluidic fittings provide a simple method for connecting capillaries or tubing to chips.

Figure 1 shows a diagram of a generic microfluidic chip. The steps below require a chip with capillary/tubing connections already in place (information on applying CapTite bonded port connectors is included on Page 3 if needed).

### Filling the Channel

1. Filter and degas your solution (water or buffer). Sonication for 5 to 10 minutes is suitable for degassing up to 10 mL volumes.
2. Referring to Figure 1, using a syringe with a one-piece fitting connected to capillary or PEEK tubing, fill from bonded port connector B.
3. Place the chip on the LabSmith SVM340 Synchronized Video Microscope using either the LabSmith integrated Bread Board (iBB) or the stage plate to secure the chip.
4. Launch LabSmith's uScope software.
5. In uScope, examine the channel for bubbles or debris. Continue to flush the channel with solution if bubbles are observed, to ensure correct electrokinetic flow.
6. Once the channel is successfully filled, twist CapTite™ reservoirs into the bonded port connectors until finger-tight.

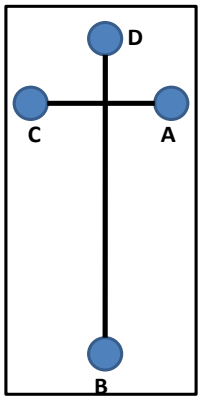


**Figure 1. Diagram of basic cross microfluidic chip. Reservoir Guide: A=Sample; B=Buffer waste; C= Sample waste; D=Buffer.**

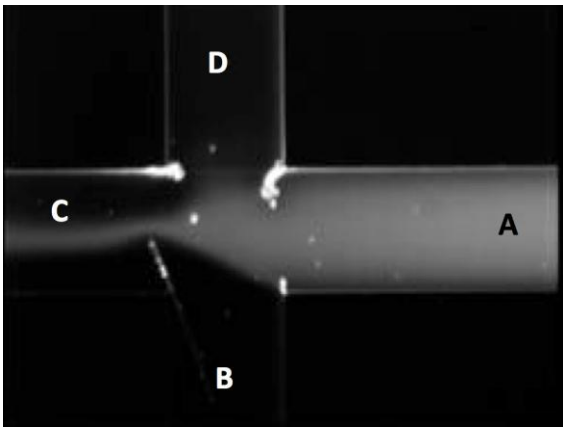


**Figure 2. Pinched injection of Oregon Green in Caliper NS 12A Chip.**

# LabSmith HVS448 Sequence Settings

7. Using an insulin syringe or syringe with needle, fill reservoirs B, C and D with buffer; fill reservoir A with the sample (in this case, Oregon Green™ or fluorescein dye).
8. Insert a piece of capillary into the reservoirs to mechanically disolodge bubbles trapped in the cone of the reservoir fitting.

## Placing Electrodes and Connecting to HVS448 Voltage Sequencer

The HVS448 High Voltage sequencer will control the electric fields. You will use four of the eight channels on the HVS448 to perform this experiment.

1. Insert platinum wire in the LabSmith microclip connectors or labeled white wire from the appropriate LabSmith High Voltage Cable. Be sure to match cable labels (**A, B, C, D**) with the terminals into which they are connected.
2. Place the electrodes in the CapTite™ reservoirs, and connect them to the terminals of the HVS. Make sure that **Terminal A** goes to sample, **B** to buffer waste, **C** to sample waste, and **D** to buffer (Figure 3).

## Verify Electrical Connections

1. Turn on the HVS448.
2. Press the **Connect** button in the Sequence toolbar.
3. Press the **Enable High Voltages** button.
4. Press **Sequence** to open the Sequence Panel.
5. Press **All** to monitor all the voltage and currents of the electrodes. Be sure that you are not getting a short in the device, and that all voltages are correct. Also note which currents are typical.

**CAUTION**: The wires are now live and exposed, at high voltage. If at any time you need to touch the electrodes or reservoirs (such as to refresh the solutions), hit the **DISABLE** button shown in Figure 4.

## Writing the Voltage Sequence Program

1. Launch LabSmith's Sequence software.
2. Choose **Tools > Simple Sequence Wizard**.
3. Under the **Step A** tab change the Step name to "Loading."
4. If you are using a standard Caliper NS12A chip with pH 7 buffer, refer to Table 1.

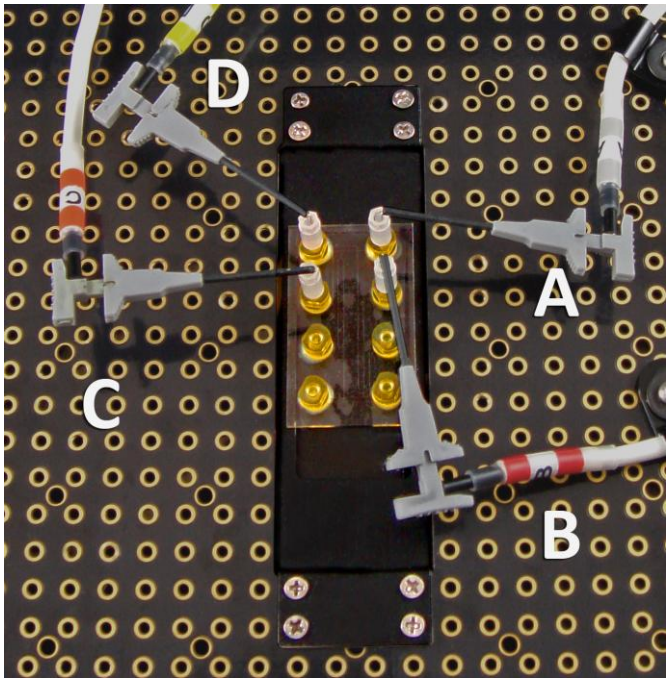


**Figure 3. Match the cables with the correct reservoirs and inputs on the HVS448.**

**Table 1. HVS Sequence Programming Voltages for Caliper NS12 A Chip**

| | Reservoir | Load V | Inject V |
|---|---|---|---|
| Sample | A | -600 | 61 |
| Buffer Waste | B | -1500 | -100 |
| Sample Waste | C | 402 | -11 |
| Buffer | D | -792 | -1500 |

5. If you are using a different chip or run buffer conditions, or if you do not get desired results, then use Sequence's manual mode do the following:
   a. In Sequence go to the **High Voltage Power Supply Monitor** mode shown in Figure 4.
   b. Adjust the applied voltage in Channels **A, B, C,** and **D** until you observe a pinched injection (Figure 2). Record and program into the Simple Sequence Wizard in the step labeled "Load". Repeat in the manual mode (Figure 3) for the run condition (sweeping injected plug to Reservoir **B**) and input those settings into the step labeled "Injection."
6. Press **Apply**.
7. Choose **File > Save As**, then name the file.
8. Now it's time to run the sequence. Press **A** to run the **Loading** voltage sequence, Press **B** to Inject (Figure 5).

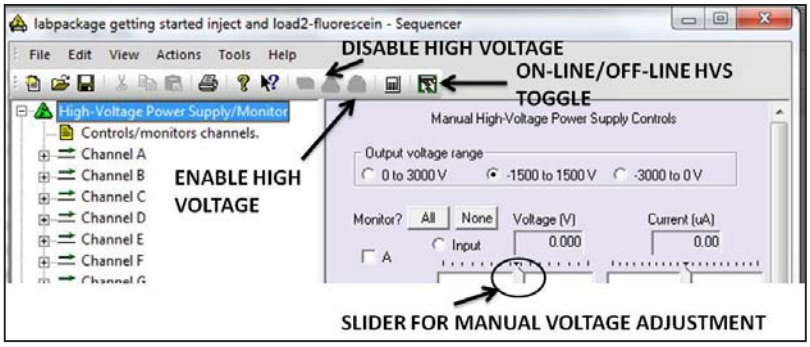


**Figure 4. Sequence Software screen showing Enable, Disable and Manual mode.**

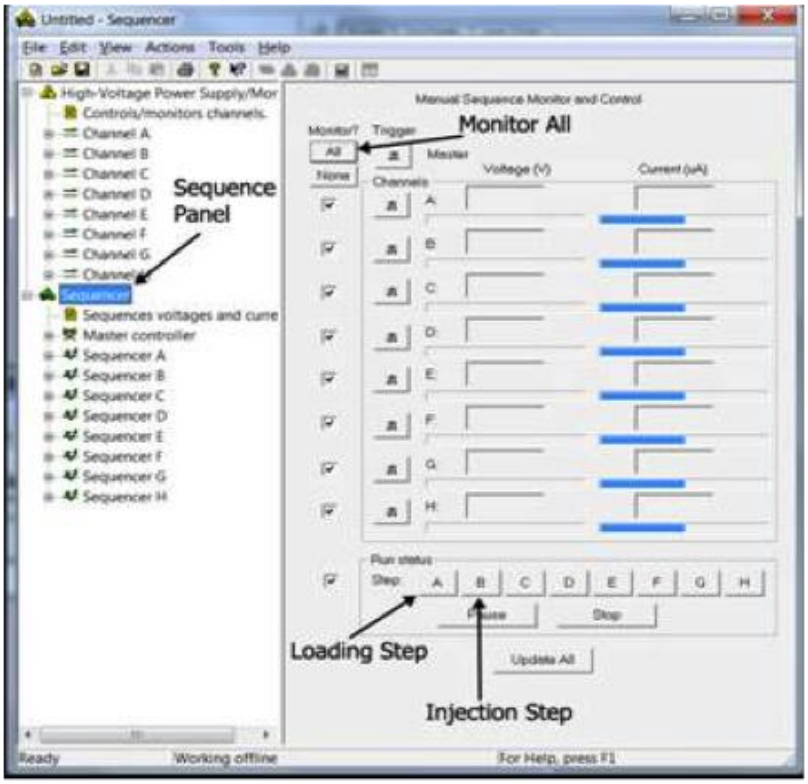


**Figure 5. Picture of Sequence Software screen when running Load and Inject sequences.**

## Mounting CapTite™ Bonded Port Connectors

1. Using the 3M DP420 two-part epoxy supplied with the LabPackage, mix two parts white base with one part amber accelerator.
2. Using a small wire or piece of fused silica capillary apply the epoxy mixture to the outer edge of the bottom of the bonded port connector (Figure 6).
3. Align centering nub with center of via hole in chip (Figure 6).
4. Press firmly. Repeat for all wells. Cure 2 hours at 60°C.

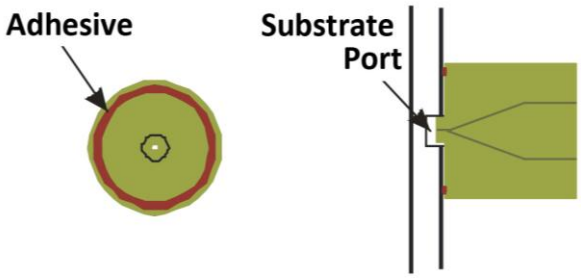


**Figure 6. Bonded port glue application and alignment.**

## Channel Cleaning

For **Polymer Chips**, flush with water then running buffer.

For **Glass Chips**, flush with 10 mM HCl, water, 10 mM NaOH, water, then running buffer.

Store cleaned chips with filtered water in channels, or air dry channels.

## Supplies

See Table 2 (this page) and Table 3 (last page) for reagents, materials, and LabSmith equipment required for a microfluidic pinched injection.

**Table 2. Reagents and Materials**

| Reagent/Supply | Source |
|---|---|
| Glass Chips | Caliper |
| Polymer Chips | microfluidic ChipShop |
| Oregon Green | Life Technologies |
| Buffer, HCl, NaOH | ThermoFisher Scientific |

## Acknowledgements

**LabSmith gratefully acknowledges Professor Sumita Pennathur of University of California Santa Barbara for the teaching laboratory procedure on which these instructions are based.**

# Lab Equipment List

## Table 3. LabSmith Equipment

| Component | Part Number | Quantity |
|---|---|---|
| **High Voltage Control (HVS448-LP)** | | |
| Eight-channel high-voltage sequencer with 3000 V maximum differential voltage | Assumes HVS448-3000D | **1** |
| High-voltage cable kit | A-HVC8-STD | **1** |
| Micro-clip set for use with high-voltage cable kit (includes 8 clips) | A-MC8-01 | **1** |
| Platinum Wire- 3 cm | A-HVPT8-STD | **1** |
| **Visualization (SVM-LP)** | | |
| Synchronous video microscope. Includes control and acquisition software, RS-170- BW camera module, LED-B illuminator module, 10X objective, and motorized X-Y focus traverse stage | SVM340 | 1 |
| Epi-fluorescent Camera for Oregon Green | EPI-Blue | 1 |
| Stainless steel sample stage for SVM340 with two rectangular openings: 20 x 32 mm and 22 x 66 mm | A-SVM-Stage | 1 |
| Integrated Bread Board | iBB | 1 |
| SVM light shield, sits on top of SVM to block ambient light | A-SHIELD | 1 |
| **Fluid Control and Connectors – specify kit for 360µm capillary** | | |
| One-Piece Fittings | C360-100 | 8 |
| One-Piece Plugs | C360-101 | 4 |
| Bonded Port Connectors* | C360-400* | 4 |
| Luer-Lock Adapters | C360-300 | 4 |
| Reservoirs | C360-RES | 4 |
| Hex wrench | LS-HEX | 1 |
| Torx wrench | LS-TORX | 1 |
| Fused silica capillary cutting stone | LS-CUTTER | 1 |
| Epoxy Adhesive* | LS-EPOXY* | 1* |
| 150µm ID fused silica capillary | 1m | 1 |

*Only included in 360µm and 1/32” kits.
Prices will vary depending on tubing size and/or substitutions.

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6111 Southfront Road, Suite E | Livermore, CA 94551 | Phone (925) 292-5161 | Fax (925) 454-9487 | www.labsmith.com | info@labsmith.com