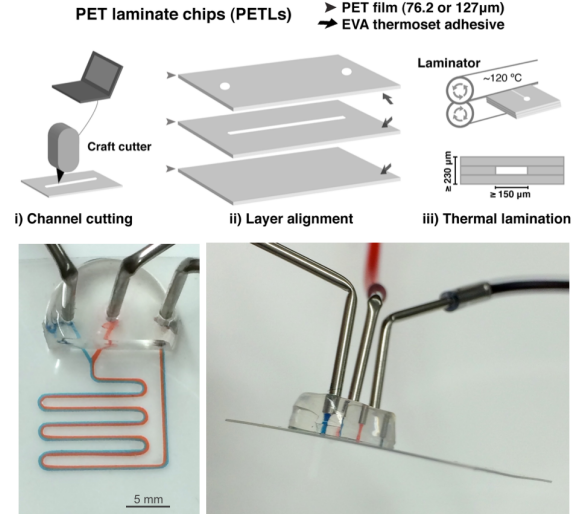


PETL FABRICATION

PETLs are microfluidic devices fabricated via thermal lamination using polyethylene terephthalate (PET) film, coated with the adhesive ethylene-vinyl acetate (EVA). These ready-to-use materials are commercially available in the form of laminating pouches of varying thickness (3 and 5 mil, or 76.2 and 127 micrometers respectively). Access to the microchannels is facilitated by inlet and outlet ports built using vinyl furniture bumpers. The devices have a thin profile, are flexible, reproducible, robust, of extremely low cost and easy to fabricate. Building a device takes under 15 minutes from design to fabrication.



Device Design

1.1 Start by sketching on a piece of paper ideas for the channels that will form part of the device.

1.2 Using any computer program that allows for drawing (e.g. Powerpoint), draw the device using lines and other shapes like curves or circles. Make lines black, solid, and with a thickness of around 4 pts.

1.3 Once the channels are finished, add a solid black circle (about 4 times thicker than the lines) to the ends of the channels that will serve as inlets and outlets. This will facilitate the loading of solutions into the channel. At this point, the overall size of the design is not critical.

1.4 The design now needs to be transferred to the Silhouette Studio software (SilSoft). Open the program and start a new document. Take a screen capture of the design and drag and drop it on the SilSoft document.

1.5 The first goal here is to trace the contours of the channels. First make sure that the image of the mat (in design view) has a grid (select the grid icon on the top right and check the Show Grid box). Now re-size the design you imported to occupy about half the size of the mat.

1.6 Now select the icon in the top right featuring a butterfly with a yellow contour. Click on the Select Trace Area icon below and use the cursor to completely select the imported design. The channels will turn yellow. Using only the Low Pass Filter settings (check box), adjust the Threshold and Scale to be accurately defined by the yellow trace. Clean, sharp yellow lines that match the design are the objective.

1.7 When the yellow trace matches the design, select the butterfly Trace icon. The channels to be cut are now shown as a red contour. If the red matches the channels, the original screen-capture of the design can be deleted.

1.8 It is time now to *size* the device. The grid on the mat shows 1 inch squares containing a total of 16 small squares each. The average device should be contained within 1 to 4 of the 1 inch squares. This will usually result in channels with a width of between 200 and 500 micrometers.

1.9 Once the size of the device is determined, position the device in the area of the mat of your preference. If many devices are printed at the same time, it helps to organize them leaving at least 1 inch of distance between devices.

1.10 In order to have access to the channels once the device is finished, a second film layer needs to be perforated at the right locations (over the circles drawn in 1.3). Using the drawing tools on the left of the window, select the ellipse and draw small circles (in red) over the inlets/outlets. When done, select the entire design (which will include the circles) and copy it (ctrl-c). Paste the design away from the original one (ctrl-p), and then select only the channels and erase them. This leaves the inlet/outlet circles in the right position with respect to each other, and should be cut in the same fashion as the channels.

1.11 Both the channel layer and the inlet/outlet layer are now ready to be cut.

Channel Cutting

2.1 Take a single layer of PET/EVA film (one layer of the laminating pouch) and lay it over the cutting mat with the smooth-reflective side facing down. The mat is adherent, use gloves to flatten out the film without introducing air bubbles. The use of gloves in this step is to avoid introducing oils and microparticles like dust found in hands.

2.2 Once the film is securely attached to the mat (make sure the area of the mat occupied by the design is the same area covered by the film), align the edge of the mat at the center of the roller in the Silhouette cutter. Press the button to load the mat.

2.3 The blade of the Silhouette cutter is manually adjusted to a number between 3 and 5. This setting depends on the film used and the condition of the mat.

2.4 Back in the SilSoft program, click on the blade icon on the top left. Select Standard and Cut Edge on the setting below. Then scroll down in the Material Type section and double click on Clear Sticker Paper. Select 4 for Speed and 10 for Thickness. All of these parameters may vary, but have worked well in the past. The Double Cut box may also be checked.

2.5 Make sure the cutter and the computer are connected. Check that the mat is properly loaded and the settings are correct. Then click on Send to Silhouette

Layer Alignment

3.1 When the cutter is finished, unload the mat. Carefully, using gloves, detach the film from the mat. Make sure not to tear the channels. The film containing the channels will leave behind (attached to the mat) the material that has been cut away. Using regular scissors cut a rectangle around the channels of the device, leaving enough space (about an inch or so) between the channels and the edge of the device.

3.2 The channel-layer is ready. The layer containing the perforations/circles corresponding to the inlets/outlets is separated from the mat in the same fashion. Clear the mat from the material left behind before reusing it.

3.3 Both the channel and the outlet/inlet layers should be superimposed (matching the ends of each channel to the perforations in the outlet/inlet layer) and cut to the same size. Cut a third piece of film to match the other 2 layers (Fig. 1a, middle).

3.4 Align the 3 layers. It is important that the smooth-reflective side of the film (the side that is not coated with EVA) in the top and bottom layers be facing outwards, not touching the channel layer. Only the opaque, EVA-coated side of the film should be in contact with the channel layer.

Thermal Lamination

4.1 Turn on the laminator. Some laminators have two settings (3 mil & 5 mil) corresponding to the thickness of the film. Select the appropriate setting and wait until the status of the laminator is Ready.

4.2 Introduce the 3-layered device, making sure that: i) no layer is significantly larger than the others and ii) the layers remain aligned during the process. To keep the layers in place, it helps to introduce a small piece of double-sided tape in between layers. Do this at the corner of the device, away from the channels. It is very important to keep any EVA coated section of the layers exposed to direct contact with the rollers inside the laminator.

4.3 The heated rollers inside the laminator (~125 Celsius) cure the adhesive (EVA), resulting in fully sealed channels with a smooth interior surface. Retrieve the device from the roller.

5. Port assembly

5.1 The device is now sealed and its thickness is equal to 3-times the height of the open channels inside. Once the device has cooled, attach vinyl bumpers to the inlets and outlets as described below (also, see Figure 1b & c).

5.2 Before attachment, bumpers are perforated with a 1 mm biopsy punch, a 19G blunt needle or a 1/32" drill bit (mounted on a dremel tool).

5.3 Detach the bumper from its base and attach it to the device. Be sure to align the perforations in the bumpers with the ports in the chip and to apply enough force to make a tight connection between the bumper and the device.

5.4 The device is now finished. Using a small capillary tube or a 18G blunt syringe and Tygon tubing, build a line to push liquids into the device. This can be done using syringe pumps or by gravity, using standard burets.

Troubleshooting

Importing design into the SilSoft document

In Apple computers one uses the key combination shift-command-4 to enter screen capture mode. Once the area of the screen is selected, a image file is generated and stored in the Document or Desktop folder. The image file can then dragged and dropped on the SilSoft window. If screen capture is not available or there is no drawing software in the computer, one can use the SilSoft drawing tools to design the channels of the device.

Tracing is inaccurate or incomplete

The software does a great job in accommodating most designs. Most difficulties occur when the thickness of the lines used to draw the channels is too thin or of low resolution. Use thicker lines and work with channel designs that are big enough to be captured at higher resolutions. A channel design can occupy an entire Powerpoint slide.

The channels are not cut all the way through

The channels are scored but when the film is detached from the mat, the channels are not open. Check that the blade is set at the proper number and that the settings for cutting (see 2.3 and 2.4) are the correct ones. Increasing the number set on the blade might help. If the problem persists, use a new blade.

The device did not come out of the laminator

The device might become stuck or attached to the rollers if one or more of the layers is larger than the others. This results in EVA curing when in direct contact with the roller and adhering to it. It is important to prevent this from occurring, since it can be very difficult to retrieve the device rendering a section of the rollers unusable.

The solution cannot be pushed into the channels

There are several problems that can lead to “clogged” devices. i) the bumper was not properly perforated: the bumper was “stabbed” instead of “hollowed out”, and the stab sealed again. Make sure the bumpers are properly perforated before attachment. ii) the bumper was attached to the wrong side of the device: because of the thinness and transparency of the devices, it is easy to confuse the top with the bottom layers. Remove the wrongly placed bumper and attach to the layer with the perforated inlet/outlets. iii) The channels are sealed shut: the lower limit achieved so far for the width of channels is approximately 150 micrometers. Attempting to cut thinner channels often results in deformation and loss of channel geometry and/or integrity. Increase the overall size of the device before cutting.

Other notes

Care should be taken when detaching the devices from the cutting mat. To prevent tears, use a flat tool (e.g. small ruler) to aid in the “lifting” of the film.

It is a good idea to use double-sided tape to keep the layers aligned during lamination.

An advantage of this method is that several devices can be cut and laminated at the same time, in batches.

The devices are ultra-thin and flexible and can withstand high flow rates without failing. However, repeated bending of the device will cause delamination.

Syringe pumps are the ideal method to push solutions into the chips. Flow rates ranging from 5 to 250 microliters/minute are used routinely. Burets can also be used, and the best results are achieved when the burets are filled with more than 20 ml of solution. Flow may stop (due to the high resistivity of the very small channels) when there is not enough pressure due to low volumes.

TASK	Equipment/Reagent	Specifications	Brand/Seller
Channel design	Computer	Drawing software (PPT, Keynote, Illustrator, etc.)	Any
Channel cutting	Craft Cutter	Silhouette Cameo	Silhouette
	Cutting mat + blade		Silhouette
	PET/EVA film	3 or 5mil laminating pouches	Scotch/3M
	Powder-free gloves		Any
Thermal lamination	Thermal laminator	Standard/basic home/office model	Scotch/3M
	Double-sided tape	Any size	Scotch/3M
	Scissors		Any
Device assembly	Vinyl furniture bumpers	Round, clear, self-adhesive (9.5mm to ½ inch diameter)	Everbilt Home Depot
	Biopsy punch (<i>optional</i>)	1mm punch	Miltex/TedPella
	Drill (<i>preferred</i>)	1/16" drill bit (we use a Dremel rotary tool)	Any
Device testing	50 ml glass burettes w/ stopcock	Regular burettes on stands	Any
	Syringe pump (<i>optional</i>)	Allows for precise control of flow (will require syringes+blunt needles, 18G)	New Era
	Capillary tubes	1-5 ul std tubes, require a tool to cut in smaller sections	VRW
	18G blunt needles 1-1/2 tip (<i>optional, replace capillary tubes</i>)	Detach luer adaptor or cut using rotary tool	Science Lab Needles
	Tygon tubing	1/32 X 3/32 (IDxOD) 1/32 wall	Cole Parmer
	Male Luer adaptor	Lock ring barb, 1/16 ID	Cole Parmer