channel **388** as well as a downstream channel **390** of the gate junction **386** can be made wide (e.g., 500 μm) and deep (e.g., 500 μm) to help ensure the wax stops at the gate junction **386**. The amount of gate material melted and moved out of the gate junction **386** may be minimized for optimal gate **380** opening. As an off-cartridge heater may be used to melt the thermally responsive substance in gate **380**, a misalignment of the heater could cause the wax in the gate loading channel **382** to be melted as well. Therefore, narrowing the dimension of the loading channel may increase reliability of gate opening. In the case of excessive amounts of wax melted at the gate junction **386** and gate loading channel **382**, the increased cross-sectional area of the downstream channel **390** adjacent to the gate junction **386** can prevent wax from clogging the downstream channel **390** during gate **380** opening. The dimensions of the upstream channel **388** at the gate junction **386** can be made similar to the downstream channel **390** to ensure correct wax loading during gate fabrication.

[0162] In various embodiments, the gate can be configured to minimize the effective area or footprint of the gate within the network and thus bent gate configurations, although not shown herein are consistent with the foregoing description.

[0163] Vents

[0164] In various embodiments, the microfluidic network can include at least one hydrophobic vent in addition to an end vent. A vent is a general outlet (hole) that may or may not be covered with a hydrophobic membrane. An exit hole is an example of a vent that need not be covered by a membrane.

[0165] A hydrophobic vent (e.g., a vent in FIG. **20**) is a structure that permits gas to exit a channel while limiting (e.g., preventing) quantities of liquid from exiting the channel. Typically, hydrophobic vents include a layer of porous hydrophobic material (e.g., a porous filter such as a porous hydrophobic membrane from GE Osmonics, Minnetonka, Minn.) that defines a wall of the channel. As described elsewhere herein, hydrophobic vents can be used to position a microdroplet of sample at a desired location within a microfluidic network.

[0166] The hydrophobic vents of the present technology are preferably constructed so that the amount of air that escapes through them is maximized while minimizing the volume of the channel below the vent surface. Accordingly, it is preferable that the vent is constructed so as to have a hydrophobic membrane **1303** of large surface area and a shallow cross section of the microchannel below the vent surface.

[0167] Hydrophobic vents are useful for bubble removal and typically have a length of at least about 2.5 mm (e.g., at least about 5 mm, at least about 7.5 mm) along a channel **1305** (see FIG. **13**). The length of the hydrophobic vent is typically at least about 5 times (e.g., at least about 10 times, at least about 20 times) larger than a depth of the channel within the hydrophobic vent. For example, in some embodiments, the channel depth within the hydrophobic vent is about 300 microns or less (e.g., about 250 microns or less, about 200 microns or less, about 150 microns or less).

[0168] The depth of the channel within the hydrophobic vent is typically about 75% or less (e.g., about 65% or less, about 60% or less) of the depth of the channel upstream 1301 and downstream (not shown) of the hydrophobic vent. For example, in some embodiments the channel depth within the hydrophobic vent is about 150 microns and the channel depth upstream and downstream of the hydrophobic vent is about 250 microns. Other dimensions are consistent with the description herein.

[0169] A width of the channel within the hydrophobic vent is typically at least about 25% wider (e.g., at least about 50% wider) than a width of the channel upstream from the vent and downstream from the vent. For example, in an exemplary embodiment, the width of the channel within the hydrophobic vent is about 400 microns, and the width of the channel upstream and downstream from the vent is about 250 microns. Other dimensions are consistent with the description herein.

[0170] The vent in FIG. **20** is shown in a linear configuration though it would be understood that it need not be so. A bent, kinked, curved, S-shaped, V-shaped, or U-shaped (as in item **208** FIG. **11**) vent is also consistent with the manner of construction and operation described herein.

Use of Cutaways in Cartridge and Substrate To Improve Rate of Cooling During PCR Cycling

[0171] During a PCR amplification of a nucleotide sample, a number of thermal cycles are carried out. For improved efficiency, the cooling between each application of heat is preferably as rapid as possible. Improved rate of cooling can be achieved with various modifications to the heating substrate and/or the cartridge, as shown in FIG. **21**.

[0172] One way to achieve rapid cooling is to cutaway portions of the microfluidic cartridge substrate, as shown in FIG. **22**A. The upper panel of FIG. **22**A is a cross-section of an exemplary microfluidic cartridge taken along the dashed line A-A' as marked on the lower panel of FIG. **22**A. PCR reaction chamber **1601**, and representative heaters **1603** are shown. Also shown are two cutaway portions, one of which labeled **1601**, that are situated alongside the heaters that are positioned along the long side of the PCR reaction chamber. Cutaway portions such as **1601** reduce the thermal mass of the cartridge, and also permit air to circulate within the cutaway portions. Both of these aspects permit heat to be conducted away quickly from the immediate vicinity of the PCR reaction chamber. Other configurations of cutouts, such as in shape, position, and number, are consistent with the present technology.

[0173] Another way to achieve rapid cooling is to cutaway portions of the heater substrate, as shown in FIG. **22**B. The lower panel of FIG. **22**B is a cross-section of an exemplary microfluidic cartridge and heater substrate taken along the dashed line A-A' as marked on the upper panel of FIG. **22**B. PCR reaction chamber **901**, and representative heaters **1003** are shown. Also shown are four cutaway portions, one of which labeled **1205**, that are situated alongside the heaters that are situated along the long side of the PCR reaction chamber. Cutaway portions such as **1205** reduce the thermal mass of the heater substrate, and also permit air to circulate within the cutaway portions. Both of these aspects permit heat to be conducted away quickly from the immediate vicinity of the PCR reaction chamber. Four separate cutaway portions are shown in FIG. **22**A so that control circuitry to the various heaters is not disrupted. Other configurations of cutouts, such as in shape, position, and number, are consistent with the present technology. These cutouts may be created by a method selected from: selective etching using wet etching processes, deep reactive ion etching, selective etching using CO_2 laser or femtosecond laser (to prevent surface cracks or stress near the surface), selective mechanical drilling, selec-