# CLAIMS

[Claim 1]

A reciprocal-flow-type nucleic acid amplification device using thermal cycling by reciprocating a sample solution comprising:

heaters capable of forming a denaturation temperature zone and an extension/annealing temperature zone;

a fluorescence detector capable of detecting movement of a sample solution between the two temperature zones that is disposed to measure fluorescence intensity at a point located between the two temperature zones;

a substrate on which a chip comprising at least one microchannel contactable to each temperature zone can be placed;

a pair of liquid delivery mechanisms that allow the sample solution to move between the two temperature zones and that are configured to be open to atmospheric pressure when air blowing is stopped, making the internal pressure of the microchannel instantaneously open to atmospheric pressure and the pressure acting on the plug-like sample solution lost, thereby stopping liquid delivery immediately, wherein the liquid delivery mechanisms are microblowers or fans; and

a control mechanism that controls driving of each liquid delivery mechanism by receiving an electrical signal relating to movement of the sample solution from the fluorescence detector, the device being capable of performing real-time PCR by measuring fluorescence intensity for each thermal cycle.

[Claim 2]

A chip for nucleic acid amplification comprising:

at least one microchannel, the microchannel comprising:

curved-microchannels each for the denaturation temperature zone and the extension/annealing temperature zone of the nucleic acid amplification device according to claim 1;

a linear intermediate-microchannel connecting the

curved-microchannels; and

connections at both ends of the microchannel, the connections being connectable to the liquid delivery mechanisms of the nucleic acid amplification device according to claim 1, wherein

a width and a depth of the microchannel are constant, and

the chip is configured so that the fluorescence intensity of a sample solution in the linear intermediate-microchannel is measurable by the fluorescence detector of the nucleic acid amplification device according to claim 1.

[Claim 3]

A nucleic acid amplification method comprising the following steps:

step 1: placing the chip for nucleic acid amplification according to claim 2 on the substrate of the nucleic acid amplification device according to claim 1 in such a manner that the denaturation temperature zone includes one curved-microchannel and the extension/annealing temperature zone includes another curved-microchannel;

step 2: introducing a sample solution into the microchannel;

step 3: connecting a pair of liquid delivery mechanisms to liquid delivery mechanism connections at both ends of the microchannel; and

step 4: reciprocating the sample solution between the two curved-microchannels of the microchannel by the liquid delivery mechanisms to perform thermal cycling, and simultaneously measuring the fluorescence intensity of the sample solution and confirming the passing of the sample solution for each thermal cycle using at least one fluorescence detector in the intermediate-microchannel to perform real-time PCR.

[Claim 4]

The nucleic acid amplification method according to claim 3, wherein the measurement of fluorescence intensity is performed by simultaneously measuring two or more fluorescent

wavelengths to simultaneously measuring real-time PCR of multiple genes in one microchannel.

[Claim 5]

The nucleic acid amplification method according to claim 3 or 4, wherein the measurement of fluorescence intensity is performed using a calibration curve obtained from the number of cycles Ct derived from a fluorescence intensity matrix per thermal cycle (a two-dimensional array of an amplification curve).

[Claim 6]

The nucleic acid amplification method according to any one of claims 3 to 5, wherein the nucleic acid amplification method is selected from the group consisting of polymerase chain reaction (PCR), reverse transcription PCR (RT-PCR), one-step RT-PCR, multiplex PCR, multiplex RT-PCR, real-time PCR, and real-time RT-PCR.

[Claim 7]

The nucleic acid amplification method according to any one of claims 3 to 6, the method comprising:

connecting an end of a filtered pipette tip of a micropipette to one of the connections so as to introduce a sample solution into the microchannel;

removing the micropipette with the pipette tip being connected to the connection, and then connecting the pipette tip to one of the liquid delivery mechanisms.

[Claim 8]

The nucleic acid amplification method according to any one of claims 3 to 7, wherein the volume of the sample solution introduced into the microchannel is in the range of 5 μL to 50 μL.

[Claim 9]

The chip for nucleic acid amplification according to claim 2, wherein the chip is made of a material selected from glass, quartz, silicon, and plastic.