

WHAT IS CLAIMED IS:

1. A method, comprising:
reacting a first solution and a different, second solution on a flow cell by flowing the first solution over an array of amplification sites on the flow cell and subsequently flowing the second solution over the array of amplification sites;
the first solution including a number of target nucleic acids and a first reagent mixture that comprises nucleoside triphosphates (NTPs) and one or more replication enzymes, the target nucleic acids in the first solution transporting to and binding to the amplification sites at a transport rate, the first reagent mixture amplifying the target nucleic acids that are bound to the amplification sites to produce clonal populations of amplicons originating from corresponding target nucleic acids, the amplicons produced at an amplification rate that exceeds the transport rate;
and the second solution including a second reagent mixture and lacking the target nucleic acids, the second solution to increase a number of the amplicons in the clonal populations at the amplification sites.
2. The method of claim 1, wherein the second reagent mixture includes the NTPs and the one or more replication enzymes of the first reagent mixture.
3. The method of claim 1, wherein the flow cell includes a plurality of primers attached to the flow cell at the amplification sites, wherein the first solution reacts on the flow cell to bind the target nucleic acids and the amplicons to a first subset of the primers, and wherein the second solution reacts on the flow cell to produce additional amplicons and bind the additional amplicons to at least some of the primers in an exposed subset of the primers different than the first subset.
4. The method of claim 1, further comprising removing the first solution from the flow cell prior to flowing the second solution over the array of amplification sites on the flow cell such that the only target nucleic acids on the flow cell as the second solution flows over the array of amplification sites are bound to the flow cell and not free-floating within the first solution.

5 5. The method of claim 1, wherein the array of amplification sites are disposed along a surface of the flow cell, wherein the first solution is flowed through an inlet port of the flow cell across the surface of the flow cell, and wherein the second solution is subsequently flowed through the inlet port across the surface of the flow cell.

10 6. The method of claim 1, wherein the flow cell includes a plurality of primers attached to the flow cell at the amplification sites, wherein at least some of the primers bind to the target nucleic acids in the first solution responsive to flowing the first solution over the array of amplification sites, and wherein subsequently flowing the second solution that lacks the target nucleic acids over the array of amplification sites results in no additional binding of the primers to the target nucleic acids.

15 7. The method of claim 1, wherein the target nucleic acids are only flowed over the array of amplification sites on the flow cell with the first solution.

8. The method of claim 1, wherein the second reagent mixture includes the NTPs and a polymerase.

20 9. The method of claim 1, wherein the second reagent mixture includes one or more of a helicase and a recombinase.

25 10. The method of claim 1, wherein the second reagent mixture includes a primer having at least one of a P5 primer sequence or a P7 primer sequence.

11. The method of claim 1, wherein the amplification sites are wells along a surface of the flow cell, the wells separated from each other by interstitial regions along the surface.

30 12. The method of claim 1, further comprising controlling the transport rate of the target nucleic acids to the amplification sites using one or more of: controlling a concentration of the target nucleic acids in the first solution, controlling a viscosity of the first solution,

controlling an average size of the target nucleic acids, and controlling a presence or absence of a molecular crowding reagent in the first solution.

13. The method of claim 1, further comprising controlling the amplification rate of the target nucleic acids using one or more of: controlling a concentration of the NTPs in the first reagent mixture, controlling a concentration of the one or more replication enzymes in the first reagent mixture, and controlling the temperature at the amplification sites.

14. The method of claim 1, wherein the first solution and the second solution are isothermally flowed over the array of amplification sites.

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15. A fluidic system, comprising:
a reagent manifold including at least one valve in fluid communication with an inlet port of a flow cell that includes an array of amplification sites, the reagent manifold further including a plurality of channels fluidly connected between the at least one valve and corresponding reagent reservoirs; and

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a controller including one or more processors, the controller to control the at least one valve and a pump to flow a first solution through the inlet port over the array of amplification sites on the flow cell and to subsequently flow a different, second solution through the inlet port over the array of amplification sites on the flow cell;

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wherein the first solution includes a number of target nucleic acids and a first reagent mixture that comprises nucleoside triphosphates (NTPs) and one or more replication enzymes, the number of target nucleic acids in the first solution exceeding a number of the amplification sites in the array, the first solution reacting on the flow cell to produce clonal populations of amplicons at the amplification sites originating from corresponding target nucleic acids, the target nucleic acids in the first solution transporting to and binding to the amplification sites at a transport rate, the first reagent mixture amplifying the target nucleic acids that are bound to the amplification sites to produce the amplicons at an amplification rate that exceeds the transport rate;

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and wherein the second solution includes a second reagent mixture and lacks the target nucleic acids, the second solution reacting on the flow cell to increase a number of amplicons in the clonal populations of amplicons at the amplification sites.

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16. The fluidic system of claim 15, wherein the second reagent mixture has the same composition as the first reagent mixture.

5 17. The fluidic system of claim 15, wherein the controller is to control the at least one valve and the pump to mix a sample template that includes the target nucleic acids with the first reagent mixture to form the first solution, and wherein the controller is to control the at least one valve and the pump to form the second solution by mixing the second reagent mixture together without mixing the sample template with the second reagent mixture.

10 18. The fluidic system of claim 15, wherein the controller is to control the at least one valve and the pump to remove the first solution from the flow cell prior to flowing the second solution over the array of amplification sites on the flow cell such that the only target nucleic acids that are present as the second solution flows over the array of amplification sites are bound to the flow cell and not free-floating within the first solution.

19. A method, comprising:

20 mixing a first reagent mixture with an amount of target nucleic acids within a reservoir to define a first solution, the first reagent mixture comprising nucleoside triphosphates (NTPs) and one or more replication enzymes;

25 flowing the first solution from the reservoir over an array of amplification sites on a flow cell, the target nucleic acids in the first solution transporting to and binding to the amplification sites at a transport rate, the first reagent mixture amplifying the target nucleic acids that are bound to the amplification sites to produce clonal populations of amplicons originating from corresponding target nucleic acids, the amplicons produced at an amplification rate that exceeds the transport rate;

30 subsequent to flowing the first solution from the reservoir, mixing a second reagent mixture within the reservoir without adding an additional amount of the target nucleic acids to the reservoir to define a second solution, the second reagent mixture comprising fresh quantities of the NTPs and the one or more replication enzymes; and

flowing the second solution from the reservoir over the array of amplification sites on the

flow cell, the second reagent mixture reacting with the amplicons to increase a number of the amplicons in the clonal populations at the amplification sites.

20. The method of claim 19, wherein the first reagent mixture and the second reagent
5 mixture both include a buffer component, the second reagent mixture including a greater quantity of the buffer component relative to the first reagent mixture to compensate for the lack of adding the additional amount of the target nucleic acids within the second solution.