CLAIMS

1. A fluidic system for increasing the number and quality of amplicons, when producing clonal populations at amplification sites, 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 wherein the flow cell includes a plurality of forwards and reverse primers attached to the flow cell at the amplification sites, the reagent manifold further including a plurality of channels fluidly connected between the at least one valve and corresponding reagent reservoirs comprising corresponding first and second solutions a first reagent reservoir comprising a 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, wherein the target nucleic acids comprise strands with adapters at the ends that are configured to bind to the corresponding forward and reverse primers;

a second reagent reservoir comprising a second solution including a second reagent mixture comprising the NTPs and one or more replication enzymes of the first reagent mixture, and grafting primers that attach to the surface of the flow cell, but lacking the target nucleic acids; and

a controller including one or more processors, the controller configured to control the at least one valve and a pump, wherein the pump is operatively coupled to the flow cell, to define the solution within the reagent manifold 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;

Wherein the flow cell is configured to linearize and remove the reverse primers from the amplification sites prior to flowing the second solution over the array of amplification sites on the flow cell;

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 bind the target nucleic acid and amplicons to a first subset of primers 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;

Linearizing and removing the reverse primers from the amplification sites prior to flowing the second solution over the array of amplification sites on the flow cell;

and wherein the second solution includes a second reagent mixture comprising the NTPs and the one or more replication enzymes of the first reagent mixture and grafting primers that attach to the surface of the flow cell, but lacks the target nucleic acids, the second solution reacting on the flow cell to:

a) Attach the grafting primers to the surface of the flow cell,
b) Produce an additional number of amplicons in the clonal populations of amplicons at the amplification sites, and
c) Bind the additional amplicons to at least some of the primers in an exposed subset of the primers different than the first subset,

Wherein the additional amplicons produced by exclusion amplification at an amplification rate exceeding the transport rate.

2. The fluidic system of claim 1, wherein the second solution comprises additional reverse primer, and

wherein the step of linearizing and removing the reverse primers includes linearizing and removing amplicons derived from the reverse primers at the amplification site.

3. The fluidic system of claim 1, wherein the step of linearizing and removing the reverse primers does not include linearizing and removing amplicons derived from the reverse primers at the amplification site.

4. The fluidic system of claim 1, wherein the grafting primers are bicyclononyne-linked forward and/or reverse primers.

5. The fluidic system of claim 1, wherein the second reagent mixture includes a greater concentration of replication enzymes, relative to the first reagent mixture.

6. The fluidic system of claim 1, wherein the controller is configured to allow controlling 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 configured to allow controlling 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.

7. The fluidic system of claim 1, wherein the controller is configured to allow controlling 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.