

[0131] 8. PreElute_Out2_Rinse_Cycle. This step fills Out2 with 25 ul (# Out2 Rinse=50) of water and then empties it.

[0132] 9. PreElute_Prime_Elution. Elution (water) is primed (Ras3B Water Prime=2) to eliminate any air bubbles and to wash processor channels.

[0133] 10. Shuttle_Elute_1. The washed and dried bead bed is disrupted and mobilized into elution water by BPump membrane pumping. The number of BPump cycles, therefore, determines the elution volume which has been set to 15 ul (BPump Out2 Mobilizer=15) in this script. The bead/RNA/water mixture is pumped into Out2.

[0134] 11. Shuttle_Elute_2. In this final step, beads and eluted RNA are separated by re-collection of beads in BPumps. In the first substep, processor channels are re-primed with water (Ras3B Water Prime=2) to remove any air bubbles or stray beads. Next, Out2 is primed (Out2 Mix Prime=2), to minimize transfer of air bubbles to BPumps. This is a fourth programmed RNA loss, as up to 1 ul out of 15 ul (6.7%) is sacrificed. Therefore, yield after all programmed losses can be as low as 93.3% of 85%=79%. Finally, bead/RNA/water mixture is pumped through BPumps to elution ports E (BPump_Elute=30). To maximize bead capture, a dwell time (EluteDwell=1500) is introduced into each pump cycle.

B. Method for Performing Enzyme Reactions

[0135] Scripts can be written to operate and/or automate the systems, devices, and methods described herein. The following is an example of a script for performing the enzyme reactions described herein.

[0136] As shown in FIG. 27, the script for the three-step Eberwine chemistry is organized into three sections for Reverse Transcription (RT), Second Strand (SS) Synthesis, and In Vitro Transcription (IVT), respectively. Each section has in common three steps: (i) buffer priming, (ii) reaction mixing, and (iii) Fluorinert insertion. Priming removes air to ensure precise volume control of mixed solutions. Fluorinert insertion, after mixing, elevates the reaction mixture into the pipette tip for best contact with the TEC-Tip Manifold, and also eliminates evaporation during extended incubations. Any inert fluid can be used in place of Fluorinert. In some embodiments, Fluorinert 77 is used. Inert fluids of low viscosity can be chosen. (Mineral oil is manually layered onto the top surface of reaction mixtures to eliminate evaporation from the top surface. Details of the enzyme reaction script are discussed below. Note that, in this script, all pump cycles are executed by chip pumps (Pump). Chip-to-chip pump rates vary from 0.55 uL to 0.70 uL per stroke. Use of layering liquids, e.g., the fluorinert or the mineral oil, can improve the reliability or reproducibility of the experiments. For example, repeated experiments can have results that are within 0.01, 0.1, 1, 2, 3, or 5 percent of each other. The standard deviation as a percent of the average value across repeated experiments can be less than about, up to about, or about 0.01, 0.1, 1, 2, 3, or 5 percent. The result can be amplification yield, array hybridization for a particular standard or entity, or any other relevant result.

[0137] 1. Prime_for_RT. RNA (Sample) and RT reaction buffer (Ras1R) are primed consecutively (# Sample RNA=2 and # Ras1R RT Buffer=1). Note each priming cycle consists of two pump strokes that direct priming waste to RasWB and RasWR, respectively. The new zero-priming manifold system ensures only 1 or 2 strokes of priming is needed to get rid of air dead volume.

[0138] 2. Mix_RT_Rxn. The 10 ul RT reaction is mixed from 5 uL total RNA and 5 uL Ambion buffer (enzymes added). RNA (Sample) and RT Reaction Buffer (Ras1R) are mixed in a 1:1 ratio into Out1. Note that the # RT Rxn Mixing=14, as opposed to 10 cycles for 10 uL. As discussed below, this is to compensate for potential losses during the enzyme reaction run.

[0139] 3. Fluorinert_Out1. Fluorinert is first primed (# Ras4R Fluorinert Prime=5), and then pumped to Out1 (# Ras4R Fluorinert Insert=30).

[0140] The reaction is now incubated at 42C for 2 hr.

[0141] 4. Prime_for_2ndStrand. RT product (Out1) and Second Strand Buffer (Ras2R) are primed consecutively (# RT Product=31 and # Ras2R Buffer=2). Each Ras2R priming cycle has two pump strokes that direct priming waste to RasWB and RasWR, respectively. Note that since the Ambion kit provides excess Second-Strand Buffer, Ras2R is primed more (compared to Ras1R) to provide for additional purging of chip channels. Each RT product (Out1) priming cycle has only one pump stroke, directed to RasWB. Note that 31 strokes (one more than the 30 strokes for inserting Fluorinert) are used to completely remove the Fluorinert spacer. This could potentially lead to the loss of some RT product, and this is why we started with excess RT reaction mixture.

[0142] 5. Mix_2ndStrand_Rxn. The 30 ul SS reaction is mixed from 10 uL RT reaction product and 20 uL Second-Strand Buffer (enzymes added). RT product (Out1) and Second-Strand buffer (Ras2R) are mixed with 23 cycles to Out2 (# Second Strand Mixing=23). Each mixing cycle consists of two pump strokes of Second-Strand Buffer and one pump stroke of RT product (mixing ratio 2:1).

[0143] 6. Fluorinert_Out2. Fluorinert is first primed (# Ras4R Fluorinert Prime=5), and then inserted into Out2 (# Ras4R Fluorinert Insert=25).

[0144] The reaction is now incubated at 16C for 1 hr, and 65C for 10 min (heat-kill).

[0145] 7. PreIVT_Empty_Out1. To ensure that Out1 is completely empty, 10 pump cycles (hardwired into the script) empty Out1 to RasW.

[0146] 8. PreIVT_Out1_Rinse_Cycle. Out1 is filled with 10 ul (# Out1 Rinse=20) water, and then emptied to RasW.

[0147] 9. Prime_for_IVT. Second-Strand product (Out2) and IVT Buffer (Ras3R) are primed consecutively (# Second Strand Product=26 and # Ras3R Buffer=3). Each Ras3R priming has two pump strokes to RasWB and RasWR, respectively. The Ambion kit provides excess Second-Strand Buffer, so Ras3R is primed more times to provide additional purging of chip channels. Each RT product (Out1) priming has only one pump stroke, directing priming waste to RasWB. Note that # Second Strand Product Prime=26 in order to completely remove the Fluorinert spacer.

[0148] 10. Mix_IVT_Rxn The 60 ul IVT reaction is mixed in Out1 from 30 uL Second-Strand reaction product and 30 uL IVT Buffer (enzymes added) with 64 cycles (# IVT Rxn Mixing=64). Mixing ration is 1:1.

[0149] 11. Fluorinert_Out1 Fluorinert is first primed (# Ras4R Fluorinert Prime=5), and then inserted into Out1 (# Ras4R Fluorinert Insert=20).

[0150] The reaction is now incubated at 40C for 2 hr.

C. Recovery of RNA using SPRI Chemistry

[0151] We obtained SpeedBeads from Seradyne, and created our own binding buffer. We used the buffer of DeAngelis et al. (Nucl. Acids Res. (1995) 23, 4742-4743) which com-