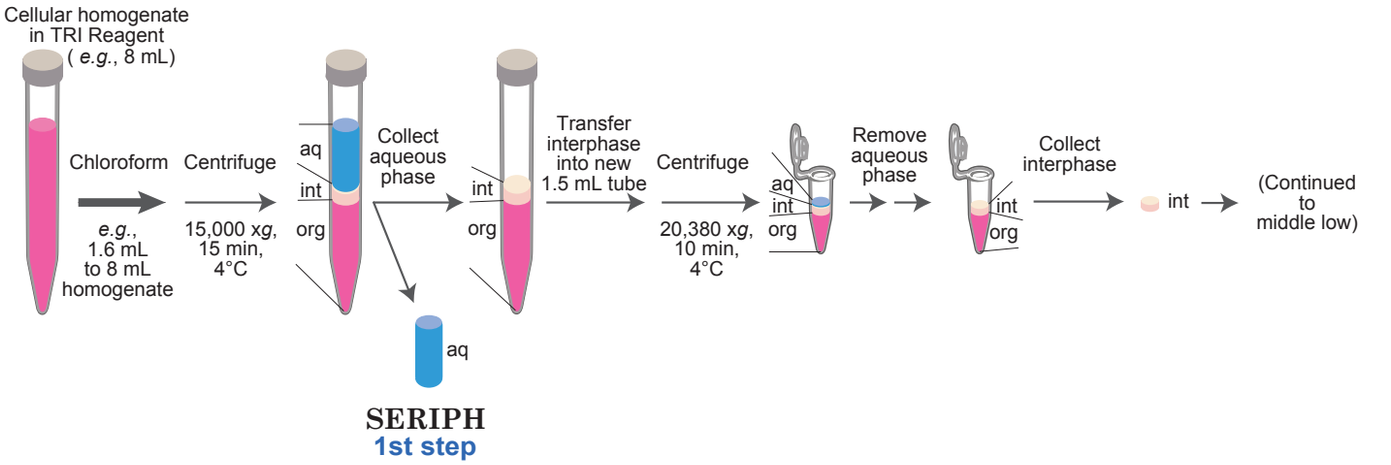

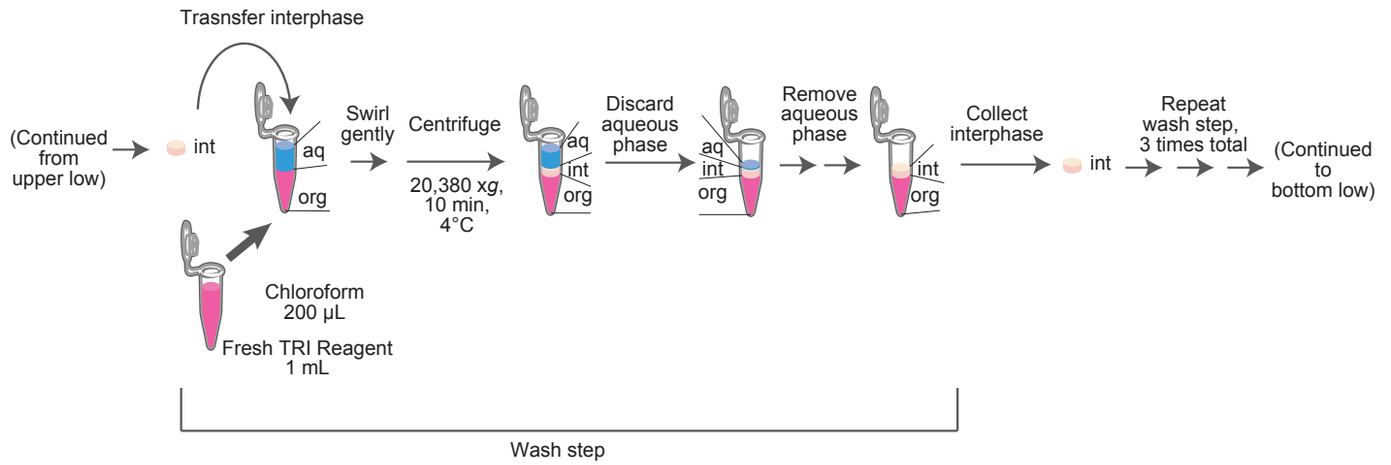

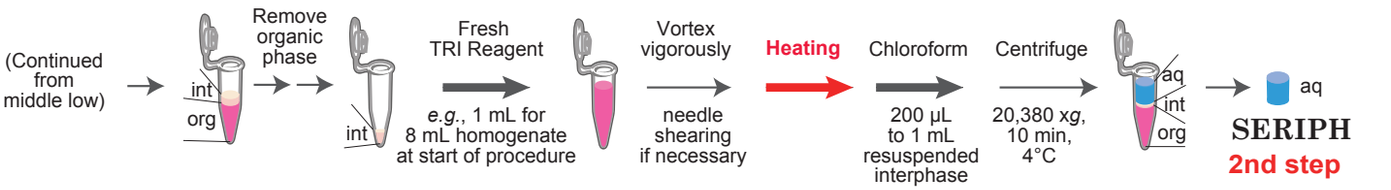

Supplemental Fig.1

Supplemental Fig S1: SERIPH Experimental Workflow

Cellular homogenate was prepared in TRI Reagent at a density of 1 × 10^6 cells/mL. In the experiments shown in Fig. 1 and Supplemental Fig. S1, 8 mL of the initial homogenate was mixed with 1.6 mL chloroform and centrifuged to induce phase separation. The upper aqueous phase containing extractable RNAs was collected as the “SERIPH 1st-step fraction.” The remaining interphase was then transferred to a 1.5-mL tube. To minimize sample loss during transfer, a small amount of the organic phase was carried over together with the interphase. The tube was centrifuged again, and the upper aqueous phase was removed as completely as possible. At this step, the organic phase was also reduced from the bottom of the tube to lower the liquid level, thereby facilitating more complete removal of the aqueous phase from the tapered bottom of the tube.
For washing, fresh TRI Reagent (1 mL) containing 200 µL chloroform was phase-separated in advance. The interphase and a small amount of the organic phase were gently overlaid onto this solution, and the tube was gently swirled to wash the interphase. After centrifugation, the aqueous phase was again removed as completely as possible. This wash step was repeated three times. In the final wash, as much of the organic phase as possible was also removed to minimize chloroform carryover into the subsequent homogenization step.

The washed interphase was resuspended in TRI Reagent and vortexed until a homogeneous lysate was obtained. For interphase derived from 8 mL of the initial homogenate, 1 mL TRI Reagent was sufficient. If aggregates could not be fully dissolved by vortexing, the sample was briefly sheared through a 20-gauge needle. The sample was then incubated at 55°C for 10 min with shaking on a bioshaker, followed by addition of chloroform (200 µL). Samples were vortexed and centrifuged at 20,380 × g for 15 min at 4°C. The resulting aqueous phase constituted the seRNA-enriched “SERIPH 2nd-step fraction.”

As shown in Supplemental Fig. S2, when SERIPH was applied to interphase material derived from hiAPGC preparation, the homogenate was first divided into multiple 1.5-mL tubes for heating on a bioshaker. The samples were then combined into a single tube, and the subsequent steps were carried out as described here.

“aq,” “int,” and “org” denote the aqueous, interphase, and organic phases, respectively.