Supplementary information

Separation and Purification of Short-, Middle-, and Long-Stranded RNAs by RP-HPLC Using Different Mobile Phases and C_{18} Columns with Various Pore Sizes

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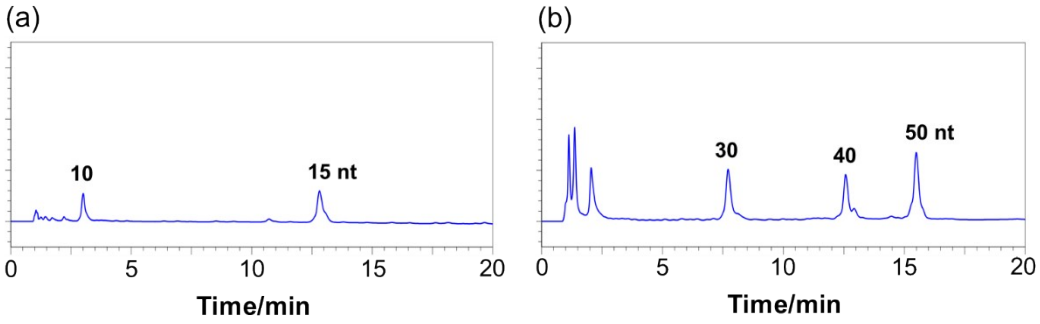

Fig. S1 HPLC chromatograms of poly($dA_{10\text{-}50}$) using a C_{18} column with normal pore. HPLC was performed using a COSMOCORE 2.6C_{18} column (2.1 mm I.D. × 100 mm) with 100 mM HFIP-15 mM HA using a linear gradient from (a) 20% to 30% (0–20 min) and 30% to 40% (0–20 min) with MeCN/MeOH (50/50, v/v) for 20 min at a flow rate of 0.2 mL/min at 65 °C. UV detection was performed at 260 nm.

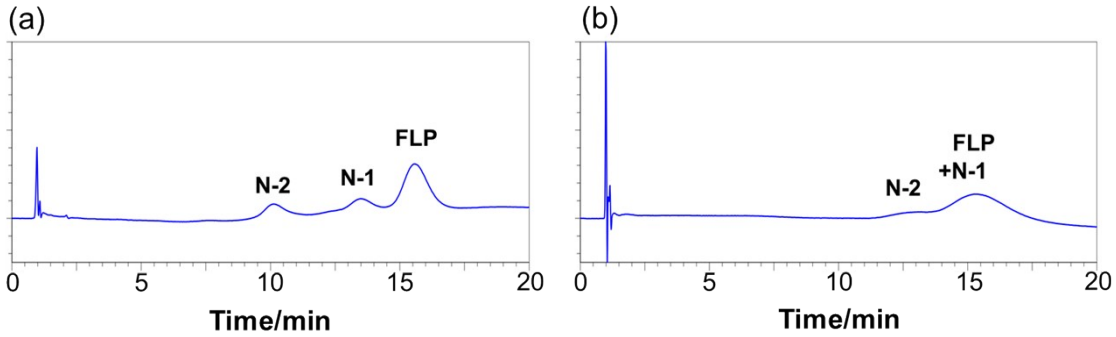

Fig. S2 HPLC chromatograms of Ampr Left2 20S (FLP), 19S (N-1), and 18S (N-2) using a C_{18} column with normal pore. HPLC was performed using a COSMOCORE 2.6C_{18} column (2.1 mm I.D. × 100 mm) with (a) 100 mM HFIP-15 mM HA and (b) 100 mM TEAA as solvent A using a linear gradient from (a) 29% to 32% (0–20 min) and (b) 33% to 39% (0–20 min) with (a) MeOH/MeCN (50/50, v/v) and (b) solvent A/MeOH/MeCN (50/25/25, v/v/v) as solvent B at a flow rate of 0.2 mL/min at 65 °C. UV detection was performed at 260 nm.

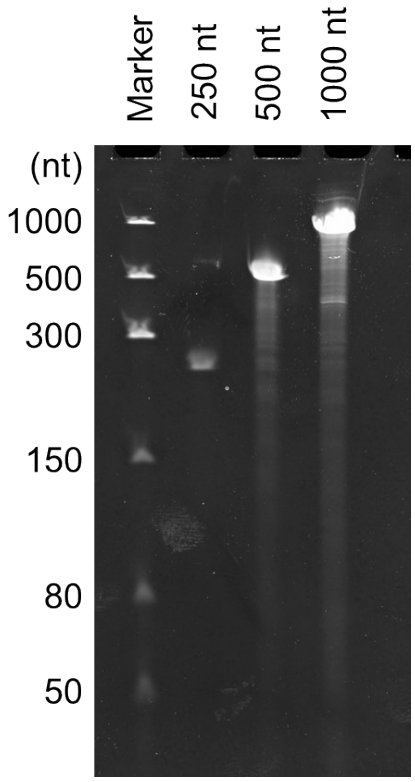

Fig. S3 Native PAGE image of the 250, 500, and 1000 nt RNAs synthesized by *in vitro* transcription.

Table S1 Half-width ($W_{1/2}$) and symmetry factor (S) values of the different mobile phases of 250, 500, and 1000 nt ssRNAs synthesized by *in vitro* transcription

	$W_{1/2\,250\,nt}$	$W_{1/2\,500\,nt}$	$W_{1/2\,1000\,nt}$	$S_{250\,nt}$	$S_{500\,nt}$	$S_{1000\,nt}$
Solvent A: TEAA-PB Solvent B: MeCN	0.21	0.17	0.18	1.54	1.22	1.45
Solvent A: HFIP-TEA-PB Solvent B: MeOH	0.33	0.35	0.34	1.02	0.86	0.90
Solvent A: TEAA Solvent B: MeCN	0.31	0.25	0.26	2.27	1.58	1.59
Solvent A: HFIP-TEA Solvent B: MeOH	0.40	0.42	0.46	1.43	1.11	1.23