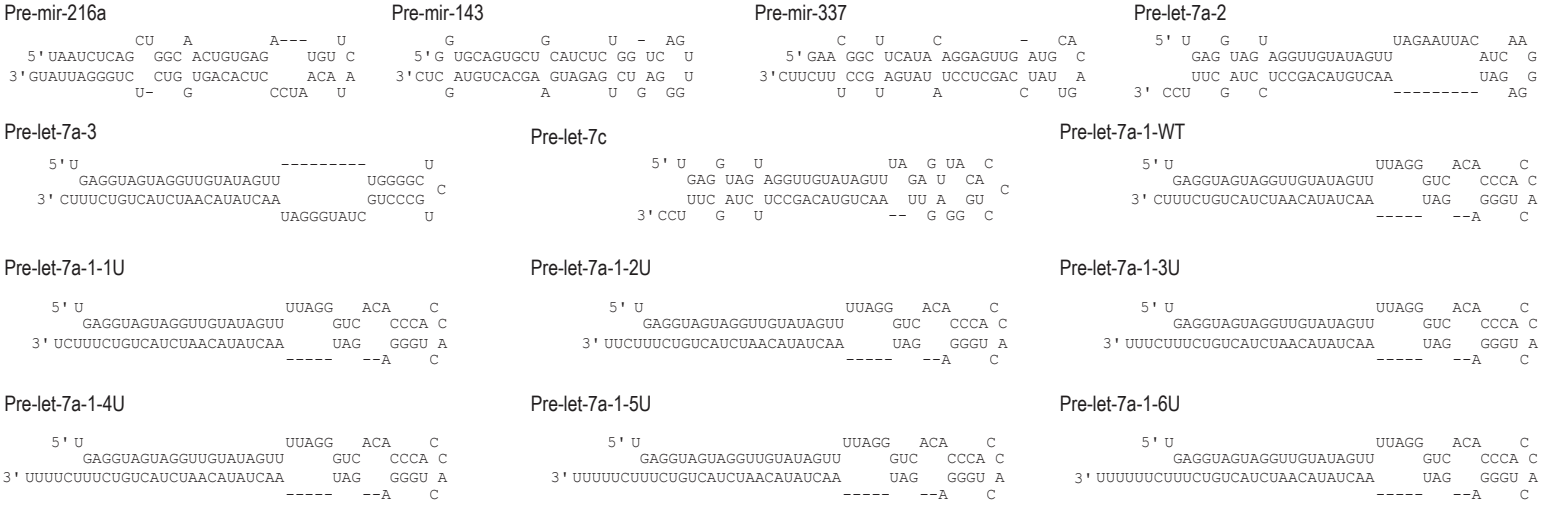
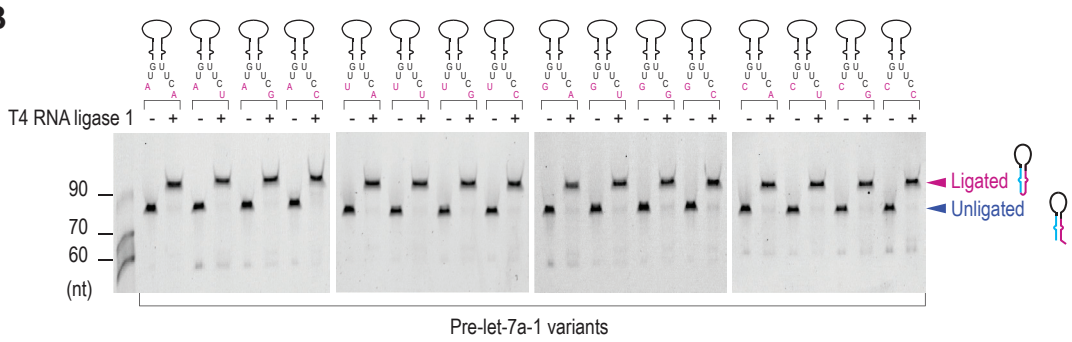
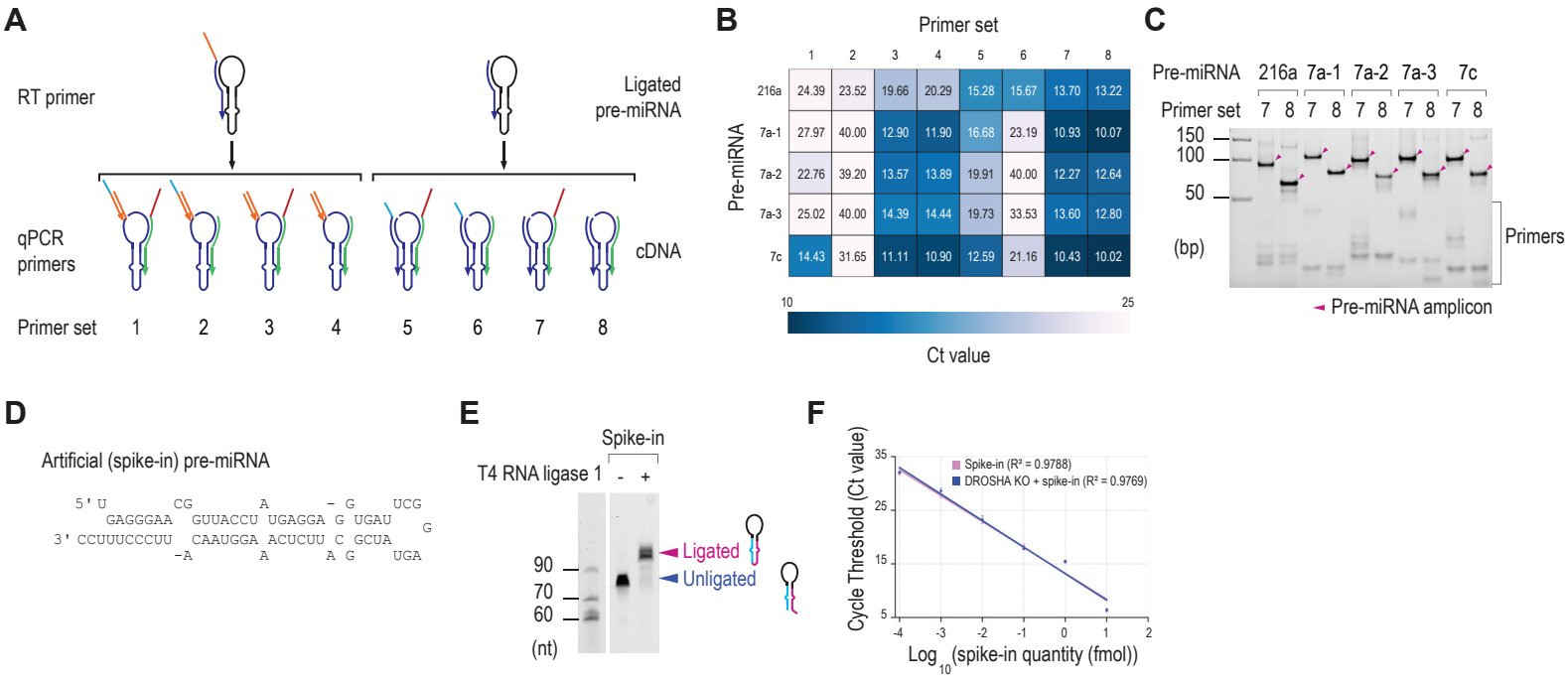
A



B



Supplemental Figure 1. Circularization of pre-miRNAs by T4 RNA ligase 1.
A) Diagram to show the sequence structures of pre-miRNAs. These sequences were folded using RNAfold (Lorenz et al. 2011).
B) Circularization of the pre-let-7a-1 variants by T4 RNA ligase 1. One pmol of each variant was incubated with 16 units of T4 RNA ligase 1 at 25°C for 2 h in the ligation buffer (NEB, M0204L) supplemented with 20% PEG 8000. The ligation reaction mixture was then analyzed in 12% denaturing-PAGE. The ligated products and original substrates were shown by the red and blue arrowheads, respectively. Primer sequences used for RNA preparation are shown in Table S1.



Supplemental Figure 2. Quantification of pre-miRNAs by iLIME-qPCR.

A) Diagram showing 8 different primer sets of RT and qPCR primers used in Fig. S2B, C. Primer sequences are shown in Table S2.

B) Primer sets 7 and 8 efficiently amplified all tested pre-miRNAs. The iLIME-qPCR experiments were carried out similarly as described in Fig. 2B, except that SYBR Green I was added to the PCR reaction. The PCR products were real-time quantified using the Roche LightCycler 480 real-time PCR system. 10 fmol of each pre-miRNA were used in each iLIME-qPCR reaction. The numbers shown on the heatmap were the average Ct values of three iLIME-qPCR repeats.

C) The iLIME-qPCR products were analyzed in native PAGE. iLIME-qPCR was conducted for 10 fmol of each pre-miRNA and stopped after 17 cycles. The amplified PCR products were then analyzed in 8% native-PAGE. The pre-miRNA amplicons were shown by the red arrowheads.

D) Diagram of the artificial (spike-in) pre-miRNA. The pre-miRNA sequences were folded using RNAfold (Lorenz et al. 2011). Primer sequences used to synthesize spike-in are shown in Table S1.

E) Circularization of the spike-in pre-miRNA by T4 RNA ligase 1. 1 pmol spike-in pre-miRNA were incubated with 16 units T4 RNA ligase 1 at 25°C for 2 h in a ligation buffer (NEB, M0204L) supplemented with 20% PEG 8000. The ligation reaction mixture was then analyzed via 12% denaturing-PAGE. The ligated products and original substrates were shown by the red and blue arrowheads, respectively.

F) iLIME-qPCR can quantify the spike-in pre-miRNA either alone or when mixed with the total RNAs isolated from DROSHA-KO cells. Different amounts (i.e., from 0.0001 fmol to 100 fmol) of the spike-in pre-miRNAs were tested. Primer sequences used spike-in quantification are shown in Table S2.

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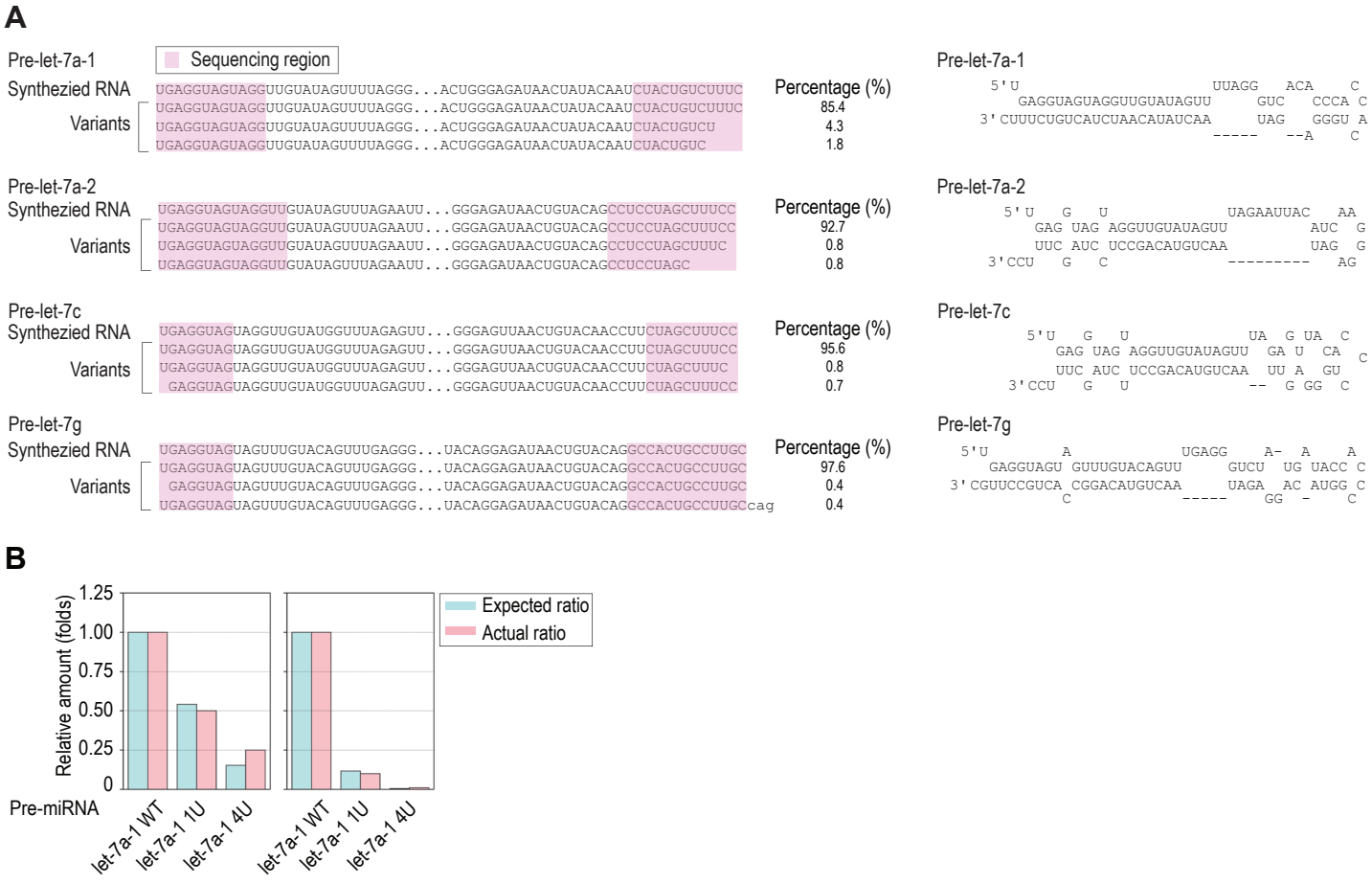
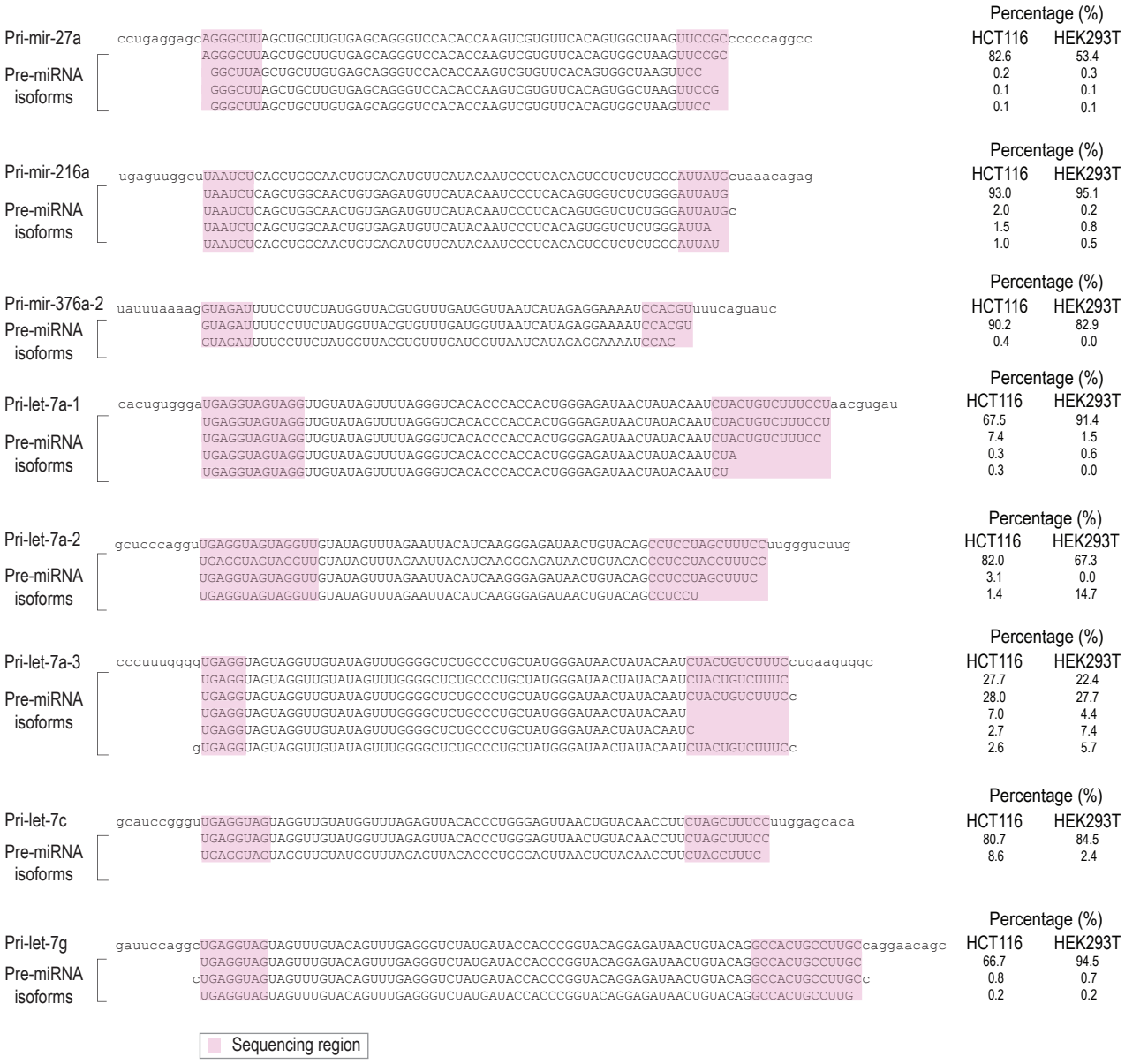
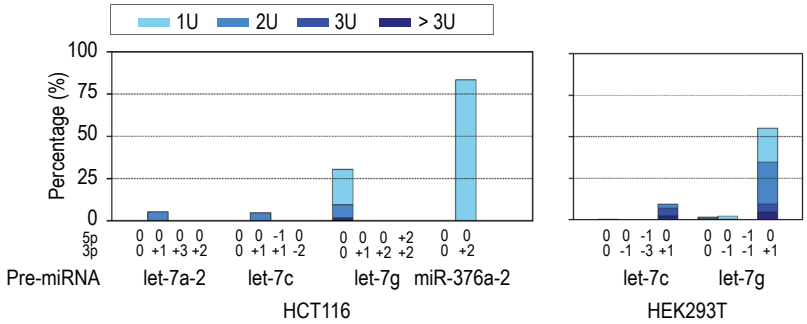


Supplemental Figure 3. Sequencing synthesized pre-miRNAs by iLIME-seq.
A) The sequences of pre-miRNAs obtained by iLIME-seq. The percentage of perfect mapped reads was estimated for each pre-miRNA.
B) iLIME-seq was conducted on mixtures of different uridylated pre-let-7a-1 variants. Thus, pre-let-7a-1, pre-let-7a-1-U, and pre-let-7a-1-4U were mixed at ratios of 1 : 0.5 : 0.25 or 1 : 0.1 : 0.01 after which they were sequenced by iLIME-seq. The ratios between the accurate pre-miRNA sequences obtained from NGS results were estimated.
Primer sequences used for RNA sequencing libraries are shown in Table S2.



Supplemental Figure 4. Sequencing of endogenous pre-miRNAs by iLIME-seq.
The percentages of different pre-miRNA isoforms identified by iLIME-seq were estimated.
Primer sequences used for RNA sequencing libraries are shown in Table S2.



Supplemental Figure 5. iLIME-seq detects 3'-end modifications in different pre-miRNA isoforms.
The 3'-end uridylation of different isoforms of the pre-miRNAs shown in Fig. 4 were sequenced by iLIME-seq. The uridylation percentages (including mono-uridylation, di-uridylation, tri-uridylation, and oligo-uridylation) were estimated for each pre-miRNA isoform. Uridylation was not detected in either pre-let-7a-2 or pre-mir-376a-2.
Primer sequences used for RNA sequencing libraries are shown in Table S2.